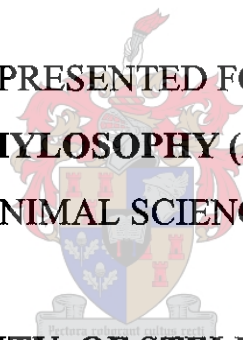


THE EFFECT OF INOCULANTS ON SILAGE FERMENTATION PROPERTIES AND ON ANIMAL PRODUCTION

by
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DISSERTATION PRESENTED FOR THE DEGREE
DOCTOR OF PHILOSOPHY (AGRICULTURE)
(ANIMAL SCIENCE)



UNIVERSITY OF STELLENBOSCH

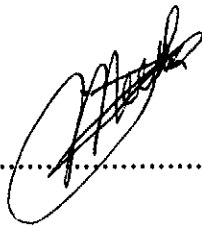
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December
2000

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

A handwritten signature in black ink, consisting of a large, stylized 'M' followed by a series of loops and a final flourish.

Date:29/11/2000.....

ABSTRACT**THE EFFECT OF INOCULANTS ON SILAGE FERMENTATION PROPERTIES
AND ON ANIMAL PRODUCTION**

by

Robin Meeske

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Maize, forage sorghum, lucerne, oats, barley and triticale are the most common silage crops in South Africa, while tropical grasses like *Eragrostis curvula* and *Digitaria eriantha* are ensiled to a lesser extent. The aim of this study was to determine the effect of adding a lactic acid bacterial inoculant to *E. curvula*, *D. eriantha*, lucerne, forage sorghum, maize and oat silage.

The effect of the addition of a lactic acid bacterial inoculant when ensiling *E. curvula* on the fermentation dynamics during ensiling and the aerobic stability of the silage was determined. The addition of the lactic acid bacterial inoculant to *E. curvula* at ensiling resulted in a more rapid lowering in pH and improved preservation. Inoculated silage had a higher lactic acid content, less protein breakdown and a lower butyric acid content compared to that of the control silage. Both silages were stable when exposed to air for five days.

Digitaria eriantha was ensiled, with or without the addition of a lactic acid bacterial inoculant containing *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with the enzymes, cellulase, hemicellulase and amylase. The addition of the inoculant resulted in a more rapid drop in pH, a higher level of lactic acid, an increase in the number of lactic acid bacteria, less protein breakdown and lower numbers of enterobacteria, clostridial spores, yeast and mould compared to the control silage. *Digitaria eriantha* hay, control and inoculated silage diets were fed to 24 Merino rams (n = 8 per treatment) to determine intake and digestibility. The intake of diets consisting of 90.9% *D. eriantha* hay, control silage or inoculated silage, differed significantly ($p < 0.05$) at 1395, 1540 and 1848 g DM/day, respectively. The *in vivo* organic matter digestibility (g/kg) of *D. eriantha* hay, untreated silage and inoculated silage diets was 561, 546, 574, respectively. The addition of the bacterial inoculant when ensiling *D.*

eriantha resulted in better preservation, improved aerobic stability, as well as a higher in vivo organic matter digestibility and intake of *D. eriantha* silage.

The addition of an inoculant or molasses to lucerne (*Medicago sativa*), ensiled in laboratory silos was investigated. The addition of the additives resulted in an increased preservation rate as indicated by a more rapid lowering of pH, a faster rate of lactic acid production and less protein breakdown compared to control silage. The inoculant was more effective than the molasses in improving the rate of preservation. The aerobic stability of lucerne silage was not affected by inoculation or the addition of molasses. The addition of an inoculant to wilted big bale lucerne silage was studied. The inoculant improved silage quality as indicated by a lower pH, higher lactic acid content, lower ammonia nitrogen content and lower level of butyric acid in inoculated silage compared to the control lucerne silage. The composition of big round bale lucerne silage differed markedly from that of lucerne ensiled in laboratory silos as the former had a higher pH, ammonia nitrogen, butyric acid and acetic acid content and a lower lactic content.

Whole crop forage sorghum cultivar FS2 was harvested at the late bloom (20.7% DM) and soft dough (28.9% DM) stages of maturity and ensiled in laboratory silos with the addition commercial silage inoculants. At both stages of maturity the inoculants caused a more rapid rate of pH decline and a higher amount of lactic acid production. All the silages were well preserved. Silages of the sorghum ensiled at the late bloom stage with all treatments were stable after 5 days of aerobic exposure, whereas sorghum ensiled at the soft dough stage with the addition of the inoculants deteriorated upon aerobic exposure. It is concluded that addition of lactic acid bacterial inoculants to mature sorghum at ensiling might impair the aerobic stability of the silage.

The yield, nutritional value and production potential of silage made from twenty one maize hybrids was compared. It was concluded that maize hybrids did differ in metabolizable energy content, rate of digestion, predicted intake and predicted milk production potential. The content of NDF and ADF did not differ between the maize hybrids used in this study and could therefore not be used to predict nutritional value or production potential.

Maize was harvested at the hard dough stage and ensiled with or without the addition of a lactic acid bacterial inoculant in laboratory silos and in 210 litre drums. The adding of the inoculant to maize at ensiling did not result in a more rapid drop in pH and higher levels of lactic acid. The intake and growth of South African Mutton Merino lambs fed inoculated and untreated maize silage diets was determined. The average daily gain of lambs fed a diet consisting of either 60% control or inoculated maize silage over a growth period of 60 days was 239 ± 26 and 255 ± 44 g/day, respectively. Although the laboratory study showed very little effect of adding a lactic acid

bacterial inoculant to maize at ensiling, lambs tended to consume more of the inoculated silage. In the second study the effect of the addition of a lactic acid bacterial inoculant with an enzyme to maize at ensiling on the fermentation dynamics during ensiling, aerobic stability of the silage, the intake, milk production and milk composition of Jersey cows fed maize silage diets was determined. The inoculant did not result in a more rapid lowering of the pH or a more rapid lactic acid production compared to untreated maize silage made in laboratory silos. Both the control and inoculated maize silages were well preserved. The addition of the inoculant to maize at ensiling improved the palatability, intake and the aerobic stability of maize silage compared to the untreated control maize silage. Milk production, milk composition, liveweight and condition score of Jersey cows was not significantly affected by the addition of the inoculant to maize silage.

The effect of the addition of an enzyme containing lactic acid bacterial inoculant to big bale oat (*Avena sativa*, cv Cederberg) silage on silage composition, silage intake, milk production and milk composition of Jersey cows was determined. The crop was cut at the bloom stage, wilted and ensiled in big round bales. The inoculant, Sil-All, was applied during the baling process on half of the bales. Silages were fed to Jersey cows in an intake and milk production study. Both the control and inoculated oat silages were well preserved. The inoculated oat silage had a lower level of butyric acid than the control oat silage. Cows fed the inoculated oat silage produced more ($P=0.05$) milk (17.7 kg/day) than cows fed the control oat silage (16.7 kg/day). The addition of a lactic acid bacterial inoculant to big bale oat silage improved silage composition and animal performance.

This study clearly showed that the composition of silages made in bunker silos under commercial farm conditions differ largely from that of silages made in small scale laboratory silos. When the effect of silage additives on aerobic stability of silage is determined the evaluation should include studies on large scale bunker silages. Evaluation of silage additives should include intake and animal production studies.

SAMEVATTING

DIE INVLOED VAN INOKULANTE OP KUILVOERFERMENTASIE- EIENSKAPPE EN OP DIEREPRODUKSIE

deur

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Mielies, voersorghum, lusern, hawer, gars en korog word algemeen as kuilvoer gewasse benut terwyl tropiese grasse soos *Eragrostis curvula* en *Digitaria eriantha* tot 'n mindere mate ingekuul word. Die doel van hierdie studie was om die invloed van 'n melksuurbakteriese-inokulant op *E. curvula*-, *D. eriantha*-, lusern-, voersorghum-, mielie- en hawerkuilvoer te bepaal.

Die invloed van 'n melksuurbakteriese-inokulant op die fermentasiedinamika en die aerobiese stabiliteit van *E. curvula*-kuilvoer is bepaal. Die toediening van die melksuurbakteriese-inokulant tot *E. curvula* tydens inkuiling het 'n vinniger tempo van pH daling en beter preserving tot gevolg gehad in vergelyking met kontrole kuilvoer. Inokulant behandelde kuilvoer het 'n hoër melksuurinhoud, minder proteïen afbraak en 'n laer bottersuurinhoud as kontrole kuilvoer gehad. Beide kuilvoere was stabiel tydens blootstelling aan lug vir vyf dae.

Digitaria eriantha is ingekuul met of sonder die toediening van 'n melksuurbakteriese-inokulant wat *Lactobacillus plantarum*, *Streptococcus faecium* en *Pediococcus acidilactici* sowel as die ensieme, sellulase, hemisellulase and amilase bevat het. Die inokulant het 'n vinniger tempo van pH-daling, hoër vlakke van melksuur en melksuurbakterië, minder proteïen afbraak en laer getalle van enterobakterië, klostridiale spore, giste and swamme in vergelyking met die kontrole tot gevolg gehad. *Digitaria eriantha* hooi, kontrole kuilvoer en geïnokuleerde kuilvoer diëte is aan 24 Merino ramme ($n = 8$ per behandeling) gevoer vir bepaling van inname en verteerbaarheid. Die inname van diëte wat uit 90.9% *D. eriantha* hooi, kontrole kuilvoer of geïnokuleerde kuilvoer bestaan het, het betekenisvol ($p < 0.05$) verskil en was 1395, 1540 en 1848

gDM/dag, respektiewelik. Die *in vivo* organiesemateriaal verteerbaarheid (g/kg) van *D. eriantha* hooi, kontrole kuilvoer en geïnkuleerde kuilvoer was 561, 546, 574, respektiewelik. Die toediening van die bakteriese-inokulant tydens inkuiling van *D. eriantha* het beter preserving, verbeterde aerobiese stabiliteit asook 'n hoër *in vivo* organiesemateriaal verteerbaarheid van *D. eriantha* kuilvoer tot gevolg gehad.

Die effek van toediening van 'n melksuurbakteriese-inokulant en van molasse tot lusern (*Medicago sativa*) ingekuul in laboratoriumsilos is ondersoek. Die inokulant toediening en molasse toediening het die tempo van preserving versnel, die pH het vinniger gedaal, melksuur is teen 'n hoër tempo geproduseer en minder proteïen afbraak het plaasgevind in vergelyking met die kontrole kuilvoer. Die tempo van preserving is meer effektief deur toediening van die inokulant verhoog as deur die toediening van molasse. Die aerobiese stabiliteit van lusernkuilvoer is nie beïnvloed deur die toediening van inokulant of molasse nie. Die effek van die toediening van 'n melksuurbakteriese-inokulant tot groot rondebtaal lusernkuilvoer is ondersoek. Die inokulant het die kwaliteit van die kuilvoer verbeter en het 'n laer pH, hoër melksuur, laer ammoniak stikstof en laer bottersuurinhoud in rondebtaal lusernkuilvoer tot gevolg gehad in vergelyking met kontrole kuilvoer. Groot rondebtaal lusernkuilvoer het grootliks verskil van lusernkuilvoer wat in laboratoriumsilos ingekuul is. Die rondebtaal kuilvoer het 'n hoër pH, hoër ammoniak-stikstof-, bottersuur- en asynsuurinhoud en 'n laer melksuurinhoud gehad as laboratorium lusernkuilvoer.

Voersorghum kultivar FS2 is op die laat blom (20.7% DM) en op die sagte deeg (28.9% DM) stadium met die byvoeging van melksuurbakteriese-inokulante ingekuul in laboratoriumsilos. Toediening van beide inokulante tot sorghum het op beide die inkuilstadiums gelei tot 'n vinniger tempo van pH daling en meer melksuurproduksie. Alle kuilvoere insluitend die kontrole kuilvoer was goed gepreserveer. Kontrole sowel geïnkuleerde sorghumkuilvoer ingekuul op die laat blomstadium was stabiel tydens aerobiese blootstelling vir 5 dae. Sorghumkuilvoer ingekuul op die sagtedeegstadium met die byvoeging van inokulante was onstabiel tydens aerobiese blootstelling. Die toediening van melksuurbakteriese-inokulante tot sorghum wat op die sagtedeegstadium ingekuul word kan aerobiese stabiliteit van die kuilvoer grootliks benadeel.

Die opbrengs, voedingswaarde en produksiepotensiaal van kuilvoer gemaak van 21 mielie hibriede is vergelyk. Verskille in metaboliseerbare energie inhoud, tempo van vertering, voorspelde inname en voorspelde melkproduksie het tussen mielie hibriede voorgekom. Die neutraalbestandevesel- en suurbestandeveselinhoud het nie verskil tussen hibriede nie en derhalwe kon dit nie gebruik word om voedingswaarde of produksiepotensiaal te beraam nie.

Mielies is op die hardedeegstadium met of sonder die toediening van 'n melksuurbakteriese-inokulant in laboratoriumsilos en 210 liter dromme ingekuul. Die toediening van die inokulant het geen invloed op tempo van pH-daling of produksie van melksuur gehad nie. Die inname en groei van SA Vleismerino lammers wat 'n dieet bestaande uit 60% kontrole of inokulant behandelde mieliekuilvoer ontvang het, is bepaal. Die gemiddelde daaglikse toename van lammers was 239 ± 26 and 255 ± 44 g/dag vir die kontrole en inokulant mieliekuilvoer dieet respektiewelik. Alhoewel die laboratoriumstudie weinig verskille tussen die kontrole en die geïnokuleerde mieliekuilvoer getoon het, het lammers geneig om meer van die geïnokuleerde mieliekuilvoer in te neem. In die tweede studie met mieliekuilvoer is die effek van toediening van 'n melksuurbakteriese-inokulant met ensieme, op die fermentasiedinamika tydens inkuiling, die aerobiese stabiliteit van mieliekuilvoer asook die inname, melkproduksie en melksamestelling van Jersey koeie bepaal. Die inokulant het nie die tempo van pH daling en produksie van melksuur verhoog nie en beide kontrole en geïnokuleerde mieliekuilvoer was goed gepreserveer. Die toediening van die inokulant tot mieliekuilvoer het die smaaklikheid, inname en die aerobiese stabiliteit van mieliekuilvoer verhoog in vergelyking met kontrole mieliekuilvoer. Melkproduksie, melksamestelling, liggaamsmassa en kondisiepunt van Jersey koeie is nie betekenisvol beïnvloed deur die toediening van die inokulant tot mieliekuilvoer nie.

Die effek van die toediening van 'n ensiem bevattende melksuurbakteriese-inokulant tot groot rondebale hawer (*Avena sativa*, cv Cederberg) kuilvoer op die samestelling van kuilvoer, kuilvoerinnome, melkproduksie en melksamestelling van Jersey koeie is bepaal. Die gewas is gesny op die blomstadium, verwelk en as rondebalekuilvoer gepreserveer. Die inokulant, Sil-All, is tydens die baalproses toegedien op die helfte van die bale. Kuilvoere is aan Jersey koeie gevoer in 'n inname en melkproduksiestudie. Beide die kontrole en geïnokuleerde hawerkuilvoer was goed gepreserveer. Die bottersuurinhoud van geïnokuleerde hawerkuilvoer was laer as die van die kontrole hawerkuilvoer. Koeie wat geïnokuleerde hawerkuilvoer gevoer is het meer ($P=0.05$) melk (17.7 kg/dag) geproduseer as koeie wat kontrole hawerkuilvoer ontvang het (16.7 kg/dag). Die toediening van 'n melksuurbakteriese-inokulant het kuilvoer kwaliteit en diereproduksie verbeter.

Hierdie studie wys duidelike verskille uit tussen kuilvoer wat in bunkersilos onder kommersiële toestande ingekuul is, en kuilvoer wat in laboratoriumsilos gemaak is. Wanneer die effek van kuilvoerbymiddels op die aerobiese stabiliteit van kuilvoer bepaal word behoort finale evaluasie gedoen te word op kuilvoer gemaak in bunkersilos soos onder plaastoestande plaasvind. Evaluasie van kuilvoerbymiddels behoort inname en diereproduksiestudies in te sluit.

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**To my parents,
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ABBREVIATIONS

ADF	Acid detergent fibre
CFU	Colony forming units
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
DOMI	Digestible organic matter intake
FBC	Fixed baling chamber
IVOMD	In vitro organic matter digestibility
LA	Lactic acid
LAB	Lactic acid bacteria
ME	Metabolisable energy
NDF	Neutral detergent fibre
SEM	Standard error of means
TN	Total nitrogen
VBC	Variable baling chamber
VFA	Volatile fatty acid
WSC	Water soluble carbohydrate

Chapter 1

General introduction

The ensiling of crops and the use of lactic acid bacterial inoculants.

1. Introduction

In South Africa summer rainfall occurs in the northern parts and winter rainfall in the southern parts. In some areas of the Southern Cape, rainfall varies from 750 to 1200mm per year and rain occurs throughout the year. Even in this area farmers need to irrigate pastures as rainfall and rain distribution varies considerably from year to year. Continuous supply of high quality roughage to dairy cows is the basis of profitable dairy farming. To achieve this, crops or grasses have to be conserved either as standing hay, hay or silage for use during the dry season. Wilkins *et al.* (1999) estimated that the global production of silage is at least 250 million tonne of dry matter per year. Many farmers find haymaking too risky and time consuming and prefer to ensile their crops. Results of recent experiments indicate that well preserved silage may give better animal performance than hay made from the same crop (Burstedt and Murphy, 1999).

Crops have been conserved for many years by man. According to Kuchler (1926), Kirstein (1963) and Schukking (1976) ensiling was practised around 3000 years ago. Old paintings found in Egypt, dating from the period about 1000 to 1500 B.C., suggest that the Egyptians were familiar with ensiling as a means of preserving crops (McDonald *et al.*, 1991). At the Fifth Ensilage Congress held in New York in January 1866, Smith of Vermont summarised the feeling of many farmers as follows : It is unnecessary to dwell on the advantages of a system of curing fodder which shall take place as independent of the weather, or which will enable us to keep two cows in place of one on our farms. Although silage has been made for so many years the challenge today is to improve preservation efficiency and nutritive value of silages (Wilkins *et al.*, 1999)

2. Principles of ensiling

The principles of ensilage are well known. The first essential objective is to achieve anaerobic conditions under which natural fermentation can take place. In practice this is achieved by consolidating and compacting the material and the sealing of the silo to prevent re-entry of air. Air that is trapped in the herbage is rapidly removed by respiratory enzymes (McDonald *et al.*, 1991). Where oxygen is in contact with herbage for a period of time, aerobic microbial activity

occurs and yeast and mould will grow. This causes the material to decay to a useless, inedible and frequently toxic product (McDonald *et al.*, 1991). Finer chopping of plant material results in improved compaction and fermentation of silage. This then improves palatability and intake of silage (Apolant and Chestnutt, 1985).

The second objective is to discourage the activities of undesirable micro-organisms such as clostridia and enterobacteria. Clostridia are present on crops and in the soil in the form of spores. Clostridia multiply under anaerobic conditions, produce butyric acid and break down amino acids resulting in silage with a poor palatability and lower nutritional value. The enterobacteria are non-spore forming, facultative anaerobes, which ferment sugars to acetic acid and other products. Enterobacteria also have the ability to degrade amino acids (McDonald *et al.*, 1991). Growth of clostridia and enterobacteria can be inhibited by lactic acid fermentation. Lactic acid bacteria are normally present on harvested crops and these organisms ferment naturally occurring sugars like glucose and fructose to mainly lactic acid. The lactic acid produced increases the hydrogen ion concentration and undissociated acids to a level at which undesirable organisms are inhibited (McDonald *et al.*, 1991). The critical pH at which growth of clostridia and enterobacteria are inhibited depends on the moisture content and the temperature. The wetter the material the lower the critical pH will be. According to Weissbach (1996) the pH required for stability of silage at 150, 250, 350 and 450g DM/ kg, is 4.10, 4.35, 4.60 and 4.85 respectively. The growth of most acid tolerant clostridia will be inhibited by a pH just below 5.0 (Jonsson, 1991). With very wet crops at a DM concentration of 150g/kg even a pH of 4 may not inhibit clostridial growth. Clostridia are very sensitive to water availability and require wet conditions for active development. Growth of clostridia can be inhibited by reducing the moisture content by wilting prior to ensiling. Lactic acid bacteria have a high tolerance to low moisture conditions and are able to dominate the fermentation of high dry matter crops (McDonald *et al.*, 1991).

Lactic acid bacteria, which are the most important species during ensiling, are usually present on grass in numbers 1000 times lower than their main competitors, fungi and enterobacteria. After ensiling, the micro-organisms capable of anaerobic growth namely, lactic acid bacteria, enterobacteria, clostridia, some *Bacillus* spp. and yeasts begin to grow and compete for available

nutrients. The first few days of ensiling are critical to the success or failure of the subsequent fermentation. Under favourable conditions lactic acid bacteria will quickly acidify the environment to such an extent that the competing organisms will not be able to survive and the end result will be a stable, low pH silage. If however the pH is not lowered quickly enough the undesirable micro-organisms, mainly enterobacteria, clostridia and yeasts will be able to compete for nutrients. This will reduce the chances of obtaining a stable silage (McDonald *et al.*, 1991)

3. Lactic acid bacteria

Lactic acid bacteria are referred to as a group of bacteria from several genera which are noted for their ability to produce lactic acid. Lactic acid bacteria seem to coexist with plants but their role on the plant surface is still unknown. They may protect plants from pathogenic micro-organisms as lactic acid bacteria are found in high numbers on damaged plant parts (McDonald *et al.*, 1991).

Lactic acid bacteria can be divided on the basis of their fermentative metabolism in two groups namely, homofermentative and heterofermentative. Homofermentative lactic acid bacteria ferment hexose exclusively to lactic acid while heterofermentative lactic acid bacteria ferment hexose to lactic acid, acetic acid, ethanol and carbon dioxide. Classification of lactic acid bacteria is based on their cell morphology as rods (*Lactobacillus*) or cocci (*Pediococci*, *Enterococci*, *Lactococci*, *Streptococci* and *Leuconostoc*).

Lactic acid bacteria have a high acid tolerance and will grow at a pH range of 4.0 to 6.8. The *Enterococci* associated with silage will initiate growth at pH 9.6 and may reduce the pH to as low as 4.0. *Pediococci* grow optimally at pH 6.5 and usually reduce the pH to below 4.0. *Lactobacilli* grow best in slightly acidic media with an initial pH of 6.4 to 4.5 but some species will decrease the pH to 3.5. *Streptococci* grow optimally at a higher pH than the *lactobacilli*. Lactic acid bacteria, which are the most important species during ensiling, are usually present on grass in numbers 1000 times lower than their main competitors, fungi and enterobacteria. After ensiling, the micro-organisms capable of anaerobic growth namely, lactic acid bacteria, enterobacteria, clostridia, some *Bacillus* spp. and yeasts begin to grow and compete for available nutrients. The

first few days of ensiling are critical to the success or failure of the subsequent fermentation. Under favourable conditions lactic acid bacteria will quickly acidify the environment to such an extent that the competing organisms will not be able to survive and the end result will be a stable, low pH silage. If however the pH is not lowered quickly enough the undesirable micro-organisms, mainly enterobacteria, clostridia and yeasts will be able to compete for nutrients. This will reduce the chances of obtaining a stable silage (McDonald *et al.*, 1991).

Many investigations have been performed on the numbers of epiphytic bacteria on silage crops. Numbers of epiphytic bacteria found on standing grass (Fenton, 1987) and lucerne (Muck, 1989) are often below 100/g of fresh material. The numbers present on the crop at ensiling are of more practical importance. After mowing, wilting and chopping increased numbers of epiphytic lactic acid bacteria are found. The numbers at ensiling range from 50 000 to 500 000 with extremes up to 10 million/g (Pitt and Leibensperger, 1987; Ruser, 1989; Rooke, 1990; Spoelstra and Hindle, 1989).

Wilting of crops is often recommended. Pahlow and Weissbach (1996) found that only 10% of the total epiphytic lactic acid bacteria on grasses can grow at a dry matter content of above 450g/kg. Osmotolerant strains of lactic acid bacteria should therefore be identified and included in inoculants added to wilted drier silage crops.

In South Africa no survey has been done to determine the numbers of epiphytic microflora present on crops prior to ensiling. It has been shown that climatic conditions do have an impact on the numbers of viable lactic acid bacteria. High numbers at ensiling positively correlate with environmental temperature and air humidity and negatively with solar radiation (Muck, 1989; Ruser, 1989). Ruser (1989) reported on a survey of 991 grass and 370 maize crops samples and found 46 to 59% of the lactic acid bacteria were homofermentative. By increasing the number of homofermentative lactic acid bacteria more efficient fermentation may take place. The effect of adding lactic acid bacterial inoculants to silage crops under local conditions in South Africa may differ from that found in Europe, as solar radiation is much higher in South Africa.

Silage additives have been developed over years to improve the nutritive value of silages and to reduce some of the risks during the ensiling process (Henderson, 1993). A silage additive should be safe to handle, reduce DM losses, improve the hygienic quality of the silage, limit secondary fermentation, improve aerobic stability, increase the nutritive value of the silage and give the farmer a return greater than the cost of the additive (Merensalmi and Virkki, 1991).

Silage additives are marketed all over the world. In France about 30 silage additives are sold and all silage additives are evaluated prior to approval by the Ministry of Agriculture (Demarquilly and Andrieu, 1996). The experimentation required to obtain full approval is carried out at INRA (Unit of feed evaluation). Silages are made using the additive submitted for approval together with a negative control (i.e. without additive) and a positive control (i.e. formic acid applied at 3.5 l/t of fresh crop for grasses and 5.0 l/t for lucerne). Silage additives are only approved if the application of the product has produced results that are at least similar or close to those obtained with the positive control. In the United Kingdom 129 silage additives are marketed and the United Kingdom Forage Additive Approval Scheme (FAAS) has been implemented (Haigh *et al.*, 1996). Evaluation of forage additives is done on a voluntary basis. According to Haigh *et al.* (1996), 45% of silage made in England and Wales is treated with silage additives. In Germany more than 50 silage additives are on the market and a voluntarily silage evaluation scheme, the DLG-Quality-Seal for additives scheme has been put in place. Additives are evaluated to determine their effect on the fermentation process, aerobic stability of silage, prevention of clostridial development in silage, effluent production, silage intake and digestibility (Honig and Pahlow, 1993; Pahlow and Honig, 1996). In South Africa no official silage evaluation or approval scheme is presently in place. Therefore there is an urgent need to evaluate silage additives under South African conditions.

Maize, forage sorghum, lucerne, oats, barley and triticale are the most common silage crops in South Africa while tropical grasses like kikuyu (*Pennisetum clandestinum*), *Eragrostis curvula* and *Digitaria eriantha* are ensiled to a lesser extent.



The aim of this study was to determine the effect of adding a lactic acid bacterial inoculant to maize, forage sorghum, lucerne, *E. curvula*, *D. eriantha* and oats.

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Chapter 2

The ensiling of tropical grasses

The effect of a lactic acid bacterial inoculant on the fermentation dynamics of *Eragrostis curvula* during ensiling

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The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of *Digitaria eriantha* silage

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Introduction

In South Africa tropical grasses like *Eragrostis curvula* and *Digitaria eriantha* are grazed by cattle and sheep. Hay is often made from these grasses during the summer months when surplus grass is available. Losses when making hay are often high due to the sudden occurrence of rain and difficult conditions for haymaking. The ensiling of tropical grasses as alternative to haymaking should therefore be considered.

Tropical grasses often do have lower levels of water soluble carbohydrates than temperate grasses. The water soluble carbohydrate content of ryegrass varies from 7.4 to 31.4% of DM compared to 3.0 to 3.5% for *Chloris gayana*, 4.5 to 6.1% for *Setaria sphacelata*, 2.7 to 3.4% for *Paspalum dilatatum* and 5.6 % for *Cenchrus ciliaris* (McDonald *et al.*, 1991). The efficient utilization of the limited amount of water soluble carbohydrates present in tropical grasses is therefore a key to successful ensiling (Murdoch, 1960). Temperate grasses contain, reducing sugars, other sugars and fructans while tropical grasses contain reducing sugars, other sugars and starch (McDonald *et al.*, 1991). The buffering capacity of tropical grasses is often high as they may contain anions (organic acid salts, orthophosphate, sulphates, nitrates and chlorides). High levels of oxalic acid have been found in some tropical grasses which increased the buffering capacity (Playne and McDonald, 1966).

Tropical grasses have a higher fibre content than temperate grasses and are therefore more difficult to compact. This may create favourable conditions for the growth of yeasts and moulds which will result in less nutrients being available for utilization by the lactic acid bacteria.

Hardy *et al.* (1990) ensiled *D. eriantha* (Smutsfinger grass) and fed the silage to beef cattle. The intake of the silage was low resulting in a low performance of beef cows. No indication was however given on the fermentation characteristics of the *D. eriantha* silage.

Preservation of *P. clandestinum* (kikuyu) was improved when the plant material was ensiled at a dry matter content of 40% compared to 25% DM (De Figueiredo and Marais, 1989). The lactic

acid production was slow, resulting in a gradual drop in pH, especially in the wetter silage. De Figueiredo and Marais (1989) did find very high levels of acetic acid (12.4% of DM) and low levels of lactic acid (0.14% of DM) in kikuyu silage made at 25% DM. They concluded that adding a readily digestible source of carbohydrates would favour lactate fermentation and result in better preservation of kikuyu silage.

Tropical grasses are not easy to ensile and very few studies on the ensiling of tropical grasses have been undertaken. In this chapter the effect of a lactic acid bacterial inoculant on ensiling of *E. curvula* and *D. eriantha* is investigated.

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Research note:**The effect of a lactic acid bacterial inoculant on the fermentation dynamics of *Eragrostis curvula* during ensiling****R Meeske and H.M. Basson**

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African Journal of Range and Forage Science, 14(3): 103-106 (1998)**Abstract**

Making good quality hay from *Eragrostis curvula* is a risky operation in many areas in South Africa due mainly to unfavourable weather conditions. Ensiling should therefore be considered as an alternative method for conserving the forage. The aim of this study was to determine if the use of a lactic acid bacterial inoculant when ensiling *E. curvula* is beneficial in terms of the fermentation dynamics during ensiling and the aerobic stability of the silage. The composition of the fresh chopped grass was $37.8 \pm 0.1\%$ dry matter, $50.8 \pm 2.3\%$ IVOMD, $5.8 \pm 0.3\%$ crude protein, $79.2 \pm 0.34\%$ NDF on a dry matter basis. *Eragrostis curvula* was ensiled in eighteen mini silos (three replications X six periods) for each of the control and inoculated treatments to follow the fermentation dynamics during ensiling. The pH of the control silage on day 1, 2, 4, 8, 16 and 122 of ensiling was 5.90, 5.08, 4.81, 4.76, 4.67, 4.33 respectively and that of the inoculated silage was 5.12, 4.34, 4.24, 4.30, 4.14 and 4.0 on the respective days. The ammonia nitrogen as percentage of total nitrogen of the control and inoculated silage differed ($P < 0.05$) and was 14.4% and 10.9% respectively. The butyric acid content of the control and inoculated silage was 2.77% and 0.16% ($P < 0.05$) and lactic acid content 2.22% and 3.15% ($P < 0.05$) respectively. Addition of the lactic acid bacterial inoculant to *E. curvula* at ensiling resulted in a more rapid drop in pH and better preservation compared to the control silage. Both silages were stable when exposed to air for five days.

Additional keywords: Bacterial additive, fermentation dynamics, silage, subtropical grass.

1. Introduction

In South Africa more than one million hectare of arable land has been identified as marginal for maize production (Schutte and du Toit 1992). In many of these areas subtropical grasses have been established. These grasses are used for grazing and hay is made in times of surplus. Hay is made during the rainy season and curing is often slow and difficult (Dannhauser *et al.* 1986). Hay-making is therefore a risky operation, often resulting in poor quality fodder. According to Van Niekerk (1982) up to 50 000 t of *Eragrostis curvula* hay are spoiled annually by unfavourable weather conditions in Natal alone.

Ensiling may be a useful alternative. However, little information is available on the ensiling of subtropical grasses. Van Niekerk (1982) compared the performance of heifers fed *E. curvula* hay and silage. The heifers lost 9.6 kg live mass on the silage and gained 30.7 kg live mass on the hay during a 107 day feeding trial. The silage was, however, made without any additives and no indication was given of how effective the grass was ensiled.

Little information is available on the epiphytic microflora and the fermentable carbohydrate content of *E. curvula*. If insufficient viable lactic acid bacteria are present on *E. curvula* at the time of ensiling, and levels of fermentable sugars are low, rapid homofermentative fermentation can not take place (Woolford 1984). This may cause a delay in the drop of the pH of the plant material after ensiling, a higher nutrient loss and consequently silage of a poor palatability. Homofermentative lactic acid bacteria can be added at ensiling to ensure that sufficient viable lactic acid bacteria are present to convert fermentable sugars to lactic acid (Woolford 1984). Enzymes are often included in commercial silage additives to increase the fermentable sugar content of the crop. This provides additional nutrients to the lactic acid bacteria (McDonald *et al.*, 1991).

The aim of the study was to determine if the use of such a bacterial inoculant when ensiling *E. curvula* is beneficial in terms of the fermentation dynamics and aerobic stability of the silage.

2. Materials and methods

Eragrostis curvula (cv Ermelo) was established in November 1992 at the Irene Animal Production Institute (28° 13'S :25° 55'E, altitude 1524m) on a Hutton (Shigalo 46 series) soil containing 25% clay. The soil depth was 140cm. The grass was fertilized with 60kg N and 12kg P per hectare on the 26th of November 1993. Rainfall during November, December and January was 110, 92 and 132 mm respectively. After six weeks of growth the grass was cut, and immediately chopped with a Feraboli 945 silage chopper (chop length 12mm). A representative sample of the fresh chopped grass was taken and frozen at - 20 °C. Forty kilogram of fresh chopped material was thoroughly mixed and divided in two portions (20 kg) which were randomly allocated to either the control or the inoculant treatments. The inoculant, Sil-All (supplied by Alltech Biotechnology Pty. Ltd.) contained *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with the enzymes, cellulase, hemicellulase and amylase. The inoculant was sprayed onto the 20 kg of chopped material to provide 10⁶ colony forming units (CFU) of lactic acid bacteria per gram of fresh material. The grass was ensiled in eighteen 1.5 litre glass jars (J. WECK, GmbH u. Co., Wehr-Ofingen, W. Germany) for each treatment to follow the fermentation dynamics during the ensiling period. Three silos were opened for each treatment on day 1, 2, 4, 8, 16 and 122 of ensiling. A representative sample from each silo was taken and frozen at -20 °C for chemical analysis at a later stage. Gas losses were determined by weighing silos at the start and at the end of the ensiling period.

At day 122 of ensiling silage was exposed to air for 5 days and the aerobic stability was determined according to the method of Ashbell *et al.* (1991). According to this method CO₂ production during the aerobic deterioration is measured as an indicator of aerobic spoilage of silage.

Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 hours. Dried silage was ground with a Wiley laboratory mill through a 1 mm sieve. The *in vitro* organic matter digestibility (IVOMD) of dried silage was determined according to Tilley and Terry (1963) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Total

nitrogen was determined by the Kjeldahl method. Water soluble carbohydrate (WSC), pH and lactic acid were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 minutes with a stomacher. Water soluble carbohydrates were determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956) and lactic acid was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acid (VFA) content of silage was determined with a Carlo Erba 4200 gas chromatograph with flame ionization detector with a 2.35 m x 3 mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H_3PO_4). The column was conditioned for 48 hours at 165°C with a nitrogen carrier gas flow of 40 ml per minute. The ammonia nitrogen content of silage was determined by homogenizing 50 g of silage in 250 ml of a 0.1N H_2SO_4 solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Mettler 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen. Least significant differences (LSD) between treatments were determined using Statgraphics (1988).

3. Results and discussion

The average chop length of the fresh chopped material was 11.94 ± 10.32 mm and the composition was $37.8 \pm 0.1\%$ DM, $50.8 \pm 2.3\%$ IVOMD, $5.8 \pm 0.3\%$ crude protein, and $79.2 \pm 0.34\%$ NDF on a dry matter basis. The change in pH, lactic acid content and WSC content of *E. curvula* during the ensiling period is presented in Figure 1, 2 and 3 respectively. The addition of a lactic acid bacterial inoculant resulted in a lower pH, a more rapid drop in pH, a more rapid and greater production of lactic acid, and a greater decline in, and lower, WSC content of the silage until day 16 of ensiling compared to the control silage. The average loss of OM in the control silage treatment was 5.6% which was higher ($P < 0.05$) than the 3.2% OM loss in the inoculated silage during the 122 days of ensiling. This corresponded with higher ($P < 0.05$) average gas loss in the control silage, viz. 3.7% of DM as compared to 1.4% of DM lost as gas in the inoculated silage.

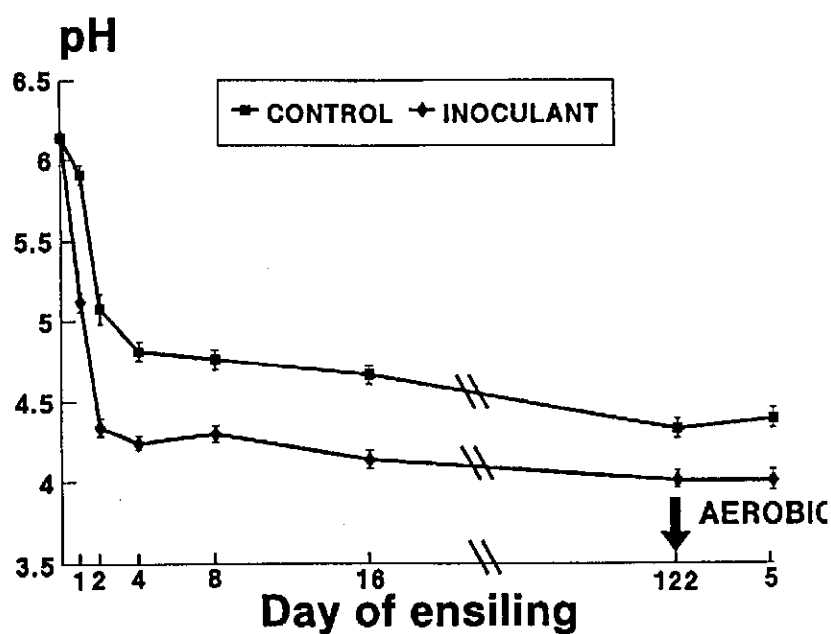


Figure 1. Changes in pH of *Eragrostis curvula* ensiled with or without the addition of an inoculant.

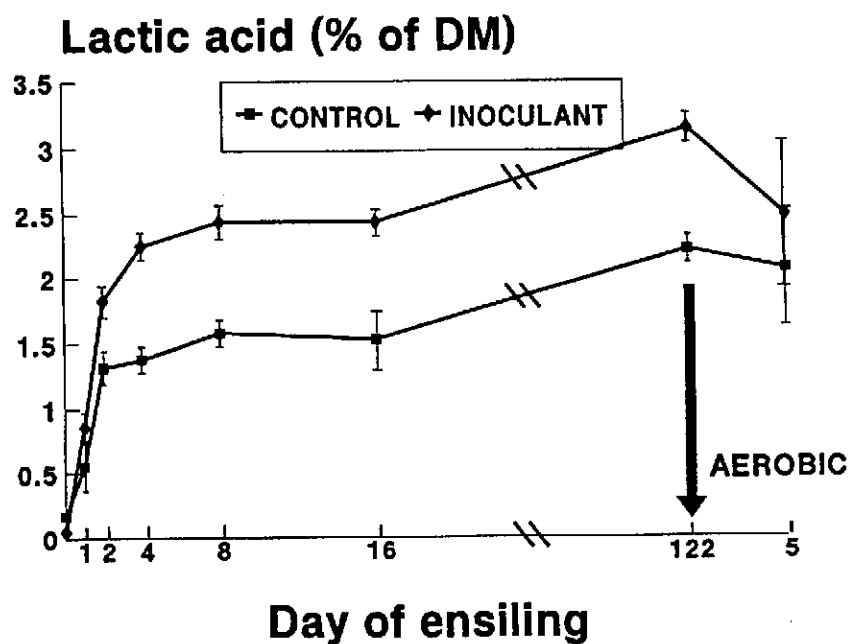


Figure 2. Changes in lactic acid of *Eragrostis curvula* ensiled with or without the addition of an inoculant.

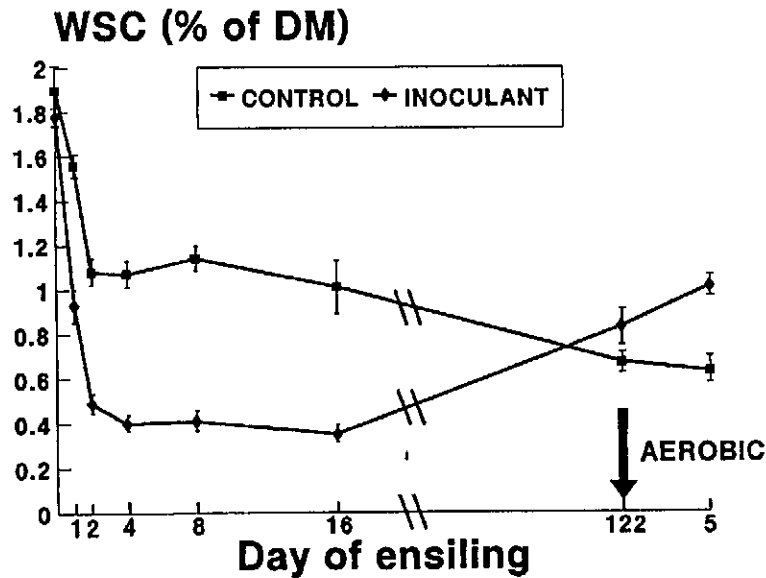


Figure 3. Changes in water soluble carbohydrates of *Eragrostis curvula* ensiled with or without the addition of an inoculant.

The composition of silage after 122 days of ensiling is presented in Table 1. There were no differences ($P>0.05$) between the control and inoculated silage in terms of dry matter, organic matter, IVOMD and nitrogen content. The addition of the inoculant to *E. curvula* resulted in silage with a lower ($P<0.05$) pH, ammonia nitrogen, butyric acid and acetic acid content and a higher lactic acid content compared to the control silage.

No CO_2 production was detected when silages were exposed to air for 5 days, indicating that both the control and the inoculated silage were stable. The minimum and maximum temperatures in the room where the aerobic stability of silage was determined was 11°C and 23°C respectively.

The addition of the inoculant resulted in increased rate of fermentation as is clearly shown in Figure 1, 2 and 3. The more rapid preservation of the inoculated silage as compared to the control silage resulted in the differences in silage composition shown in Table 1. The slower rate of pH drop in the control silage may have allowed more time for the growth of clostridia as indicated by the higher fraction of ammonia nitrogen together with a higher level of butyric acid (Table 1).

Table 1 Composition (% of DM) and standard errors of the means (SEM) of control and inoculated *Eragrostis curvula* silage after 122 days of ensiling (NF = not found). Means preceded by different superscripts in the same row differ significantly ($P < 0.05$)

Constituent	Control	Inoculant	SEM
Dry matter	36.3	36.9	0.40
Organic matter	94.8	94.7	0.10
NDF	^a 77.2	^b 76.3	0.10
IVOMD	46.3	47.2	1.30
Crude protein	5.2	5.2	0.06
NH ₃ -N (% of TN)	^a 14.4	^b 10.9	0.40
pH	^a 4.33	^b 4.00	0.01
Lactic acid	^a 2.22	^b 3.15	0.42
Acetic acid	^a 4.33	^b 4.00	0.01
N-Butyric acid	^a 2.77	^b 0.12	0.09
Iso-Butyric acid	0.01	NF	
Propionic acid	0.02	NF	
Isovaleric acid	0.07	NF	
n-Valeric acid	0.14	NF	

The growth of most acid-tolerant clostridia is inhibited by a pH just below 5 in silage with DM content of 250 g/kg. However if the DM content is 200 g/kg *Clostridia tyrobutyricum* is able to grow at pH 4.4 to 4.9 (Jonsson, 1991). Jonsson *et al.* (1990) found that although a dry matter of 350 g DM/kg suppressed clostridial activity in big bale silage, a DM of above 400 g DM/kg was required for complete inhibition. In the current study the DM content of the control silage was 363 g DM/kg and the pH after two days of ensiling was still higher than 5. This would allow growth of clostridia to take place.

The water soluble carbohydrate content of tropical grasses are generally lower than those of

temperate species (McDonald *et al.* 1991). The very low water soluble carbohydrate content of the grass at ensiling (<20g WSC/kg DM) may have led to unwanted fermentation properties in the control silage. It has been shown that grasses of tropical and subtropical origin accumulate starches instead of fructans in their vegetative tissues (Smith 1973). These starches are made up of amylose and amylopectin. Therefore the amylase added to the inoculant could have caused an increase in water soluble carbohydrates in the treated silage. The increase in WSC found in the inoculated silage from day 16 to day 122 of ensiling (Figure 3) and the significant lower NDF content (Table 2) of the inoculant treated silage compared to the control silage on day 122 of ensiling may be attributed to the WSC released by the effect of the enzymes added to the inoculum. Silage additives with enzymes should be more effective than additives without enzymes when applied to tropical grasses. When water soluble carbohydrates are limiting, lactic acid bacteria will produce less lactic acid and more acetic acid, as was found in this study (Table 1). The bacteria will have low intracellular concentrations of fructose-1,6-diphosphate which is an essential activator of lactate dehydrogenase (McDonald *et al.*, 1991) and consequently lactic acid production is limited.

It is possible that up to fifteen percent of the initial energy content of an ensiled crop may be lost due to aerobic deterioration (McDonald, 1981). The aerobic stability of silage can possibly be adversely affected by the addition of bacterial inoculants (Kung *et al.* 1991, Weinberg *et al.* 1993). In the current study both the control and inoculated silage were stable after 5 days under aerobic conditions as indicated by no production of CO₂ and no pH increase. This can be ascribed to the high acetic acid and low WSC content of both control and inoculated silage (Wolthuisen *et al.* 1989).

4. Conclusions

The addition of the lactic acid bacterial inoculant and enzymes to *E. curvula* resulted in an improvement in the fermentation dynamics during ensiling. The inoculated silage was better preserved than the control silage. Both the control and the inoculated silages were stable when exposed to air.

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The effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics, intake and digestibility of *Digitaria eriantha* silage

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Abstract

The aim of this study was to determine the effect of applying a bacterial inoculant with enzymes to *Digitaria eriantha* on the fermentation dynamics during ensiling, the aerobic stability, intake and digestibility of the silage. The grass was ensiled, with or without the addition of a lactic acid bacterial inoculant at 10^6 colony forming units per gram of fresh material, in Cryovac barrier bags. The inoculant Sil-All (Supplied by Alltech Biotechnology Pty. Ltd.) contained *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with the enzymes, cellulase, hemicellulase and amylase. To follow the fermentation dynamics during ensiling samples were taken on days 1, 2, 5, 9 and 44 of ensiling for chemical and microbiological analysis. The inoculant resulted in a more rapid drop in pH, a higher level of lactic acid and lactic acid bacteria, less protein breakdown and lower numbers of enterobacteria, clostridial spores, yeast and mould compared to control silage. Silage was made on a larger scale in two tower silos (1.5 t silage capacity) for each of the control and inoculated treatment. *D. eriantha* hay was also made at the same time. Silage and hay diets were fed to 24 Merino rams ($n = 8$ per treatment), weighing 62 ± 2.7 kg to determine intake and digestibility. The intake of diets consisting of 90.9% *D. eriantha* hay, control silage or inoculated silage, differed significantly ($p < 0.05$) at 1395, 1540 and 1848 g DM day⁻¹, respectively. The *in vivo* organic matter digestibility (g kg⁻¹) of *D. eriantha* hay, untreated silage and inoculated silage diets was 561, 546, 574, respectively. The addition of the bacterial inoculant when ensiling *D. eriantha* resulted in better preservation, improved aerobic

stability, as well as a higher *in vivo* organic matter digestibility and intake of *D. eriantha* silage.

Keywords: Inoculant; Ensiling; Hay; Preservation; Silage; Tropical grass

1. Introduction

In South Africa more than 1×10^6 ha of arable land have been identified as marginal for maize cropping (Schutte and du Toit, 1992). In many of these areas Smuts-finger grass (*Digitaria eriantha*) pastures have been established. The grass is a tufted subtropical perennial, adapted to a wide range of climatic and soil conditions (Hardy *et al.*, 1990). It is grazed by cattle and sheep and farmers often make hay when surplus plant material is available. Because hay is made during the rainy season and cuts are often heavy and leafy, curing is slow and difficult (Dannhauser *et al.*, 1986). Hay making is therefore a risky operation often resulting in poor quality fodder. Very little information is available on the ensiling of *D. eriantha*. Hardy *et al.* (1990) found that the intake of *D. eriantha* silage, made without additives, by Hereford cows amounted to 2.2% of liveweight. Although the quality of preservation was not mentioned, the observed intake could be considered as low. Van Niekerk (1982) compared performance of heifers fed subtropical grass silage (*Eragrostis curvula*) and hay made from the same sward. Heifers lost 9.6 kg on the silage and gained 30.7 kg on the hay during a 107 day feeding trial. Meeske and Basson (1998a) found that applying a lactic acid bacterial inoculant to *E. curvula* (Weeping love grass) at ensiling resulted in better preservation and improved aerobic stability of the silage.

No information on the epiphytic microflora on *D. eriantha* is presently available. Insufficient viable lactic acid bacteria on the grass at ensiling could result in a delay in the drop of pH, higher nutrient losses and silage of a poorer palatability (Woolford, 1984). The aim of this study was to determine the effect of a lactic acid bacterial inoculant with enzymes on the fermentation dynamics during ensiling, aerobic stability, intake and digestibility of *D. eriantha* silage.

2. Materials and methods

Four hectares of *D. eriantha* were established in 1989 at the Irene Animal Nutrition and Products Institute (longitude 28° 13'S, latitude 25° 55'E, altitude 1524 m) on a Hutton (series Shigalo 46) soil containing 25% clay. Fertilizer was applied at 40 kg N and 9 kg P per hectare on the 10th of December 1991 and the grass was harvested at the early flowering stage on the 22nd of January 1992.

2.1. Laboratory study

The grass was cut, wilted for 3 h, chopped (21.5 ± 16.8 mm chop length, $n = 40$) with a Feraboli 945 silage chopper, and ensiled within 3 h in Cryovac barrier bags. The grass was ensiled either without any additives or with the addition of a bacterial inoculant to provide 10^6 colony forming units (CFU) of lactic acid bacteria (LAB) per gram of fresh material. The inoculant (Sil-All, supplied by Alltech Biotechnology Pty. Ltd.) contained *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with the enzymes, cellulase, hemicellulase and amylase. Fifteen bags were filled with 1000 g of chopped material for each treatment and stored between $22.5 \pm 20^\circ\text{C}$ and $26.6 \pm 10^\circ\text{C}$. Three bags in each treatment were opened on each of days 1, 2, 5, 9 and 44 of ensiling and representative samples were taken for chemical (stored at 20°C) and microbiological analysis. At day 44 of ensiling silage was exposed to air for five days and the aerobic stability determined according to the method of Ashbell *et al.* (1991). According to this method CO_2 production is measured as an indicator of aerobic deterioration of silage.

Microbiological analyses were carried out on a composite sample of the three replicates for each of the control and treated silage for each of the test days. Microbial analyses on fresh plant material before ensiling, was done after the additive was applied. Forty grams of material was weighed into sterile stomacher bags, 360 ml of sterile saline water added and the samples were homogenized by stomaching for 3 min. The extract was further diluted. Enumeration of lactobacilli was done using Rogosa agar (Rogosa *et al.*, 1951; Oxoid CM 627) supplemented with 0.4 g l^{-1} of cycloheximide to inhibit yeast growth. Agar plates were incubated at 37°C for 72 h.

Enterobacteria were enumerated on Violet Red Bile Glucose agar (Oxoid CM 684) using the double layer technique (incubated at 37°C for 24 h). Yeasts and moulds were enumerated on malt extract agar (Difco) adjusted to pH 3.5 by the addition of 50 ml of 10% lactic acid per litre (incubated at 25°C for 72 h). Colonies were counted directly on the agar plates. Clostridial spores were determined by the most probable number technique on lactate-acetate agar as described by Pahlow (1986).

Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 h. Water soluble carbohydrates (WSC), pH and lactic acid (LA) were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 mm with a stomacher. WSC were determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956) and LA was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acids (VFA) were determined with a Carlo-Erba 4200 gas chromatograph with flame ionisation detector with a 2.35 m x 3 mm stainless-steel column packed with 10% SP 1200 containing 1% *ortho*-phosphoric acid (H_3PO_4). The column was conditioned for 48 h at 165°C with a nitrogen carrier gas flow of 40 ml per minute. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Total nitrogen was determined by the Kjeldahl method. The ammonia nitrogen content of silage was determined by homogenizing 50 g of silage in 250 ml of a 0.1 N H_2SO_4 solution for 3 minutes. The homogenate was filtered through Whatman No. 4 filter paper and the ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Mettöhm 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen. Least significant differences between treatments were determined using the Statgraphics (1988) statistical computer programme.

2.2. Intake and digestibility study

The grass was ensiled with or without inoculant and hay was made at the same time. The grass was cut at 8:30 and wilted for 3 h. Control silage, inoculated silage and hay were made by

randomly allocating wind rows to the different treatments. Grass was chopped with a Feraboli 945 chopper and two small tower silos (1.5 t silage capacity) were filled for each treatment. The inoculant was applied at 10 g per tonne of fresh chopped material by spraying 4 litre per tonne at the pickup of the silage chopper (106 CFU of LAB per gram of fresh material). The hay was baled and stored in a barn. The silage and hay were stored for a period of 15 months. Two representative samples of each silo were taken and analysed as described for the laboratory trial.

Twenty four Merino rams (62 ± 2.7 kg) were randomly allocated to three treatments. The rams were fed a diet consisting of either 90.9% *D. eriantha* hay, control silage or inoculated silage plus 3% molasses, 3% fishmeal, 0.5% urea, 1% dicalcium phosphate, 1% salt, 0.5% ammonium sulphate and 0.1% of a mineral/vitamin premix on a dry matter basis. The premix provided vitamin A, 5×10^6 IU; vitamin D3, 5×10^5 IU; vitamin E, 4000 IU; Mn, 30 g; Zn, 50 g; Co, 1 g; I, 1 g; Fe, 50 g; Mg, 250 g and Se, 0.15 g per tonne dry matter of total diet. The hay was milled with a hammermill through a 25 mm sieve. The diets were mixed and stored at -20°C . Diets were taken out of the freezer 24 h before being fed to the sheep. The diets were fed *ad lib.* plus 10% for an adaptation period of 21 days followed by a collection period of 10 days. Sheep were housed in metabolism crates. Daily intake of diets and excretion of faeces were monitored and apparent digestibility of organic matter was determined.

3. Results

3.1. Laboratory study

The DM content of the grass at ensiling was 388 ± 11 g kg⁻¹, IVOMD was 588 ± 20 g kg⁻¹ DM, the crude protein 60 ± 9 g kg⁻¹ DM, the NDF 713 ± 7 g kg⁻¹ DM and the WSC content of grass was 27.7 ± 9 g kg⁻¹ DM on a dry matter basis. The change in pH, WSC and LA of *D. eriantha* during the ensiling period is given in Figs. 1, 2 and 3, respectively. The addition of the inoculant resulted in a more rapid drop in pH, a higher production of LA, with no difference in the utilization of WSC. The LA increased more rapidly in the treated silage than in the control silage. After five days of ensiling the LA content of the inoculated silage was 17 g kg⁻¹ DM, which was

higher ($p < 0.05$) than the 3.6 g kg^{-1} DM of the control silage.

The chemical composition of silage after 44 days of ensiling is given in Table 1.

The amount of CO_2 detected after five days of aerobic exposure was 1.78 and $0.95 \text{ g CO}_2 \text{ kg}^{-1}$ DM for the control and inoculated silage, respectively ($\text{SEM} = 0.41$, $p = 0.23$). The IVOMD after five days of aerobic exposure was 49.8% and 52.2% ($\text{SEM} = 0.91$) for the control and inoculant treatment, respectively.

The counts of CFU of lactic acid bacteria, enterobacteria, yeasts and moulds and most probable number of clostridial spores are given in Table 2. The inoculated grass contained higher numbers of LAB and lower numbers of enterobacteria, yeast, mould and clostridial spores than the untreated grass silage.

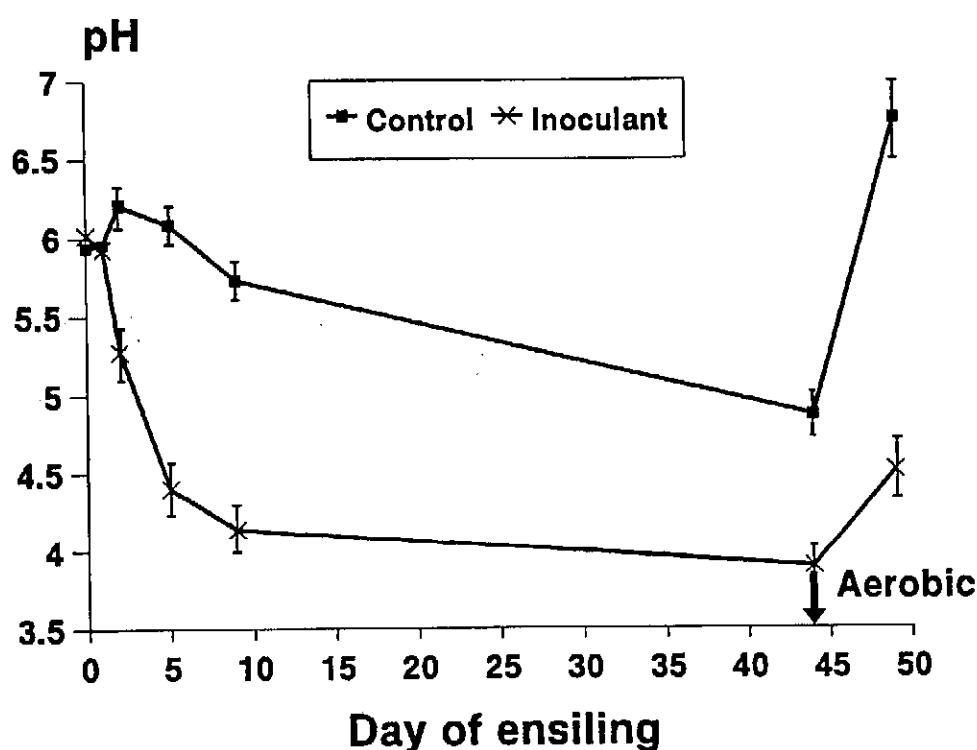


Figure 1. The change in pH of *D. eriantha* silage ensiled with or without a lactic acid bacterial inoculant. $\text{SEM} = 0.18$ (Standard error bars are shown where significant differences ($p < 0.05$) were found).

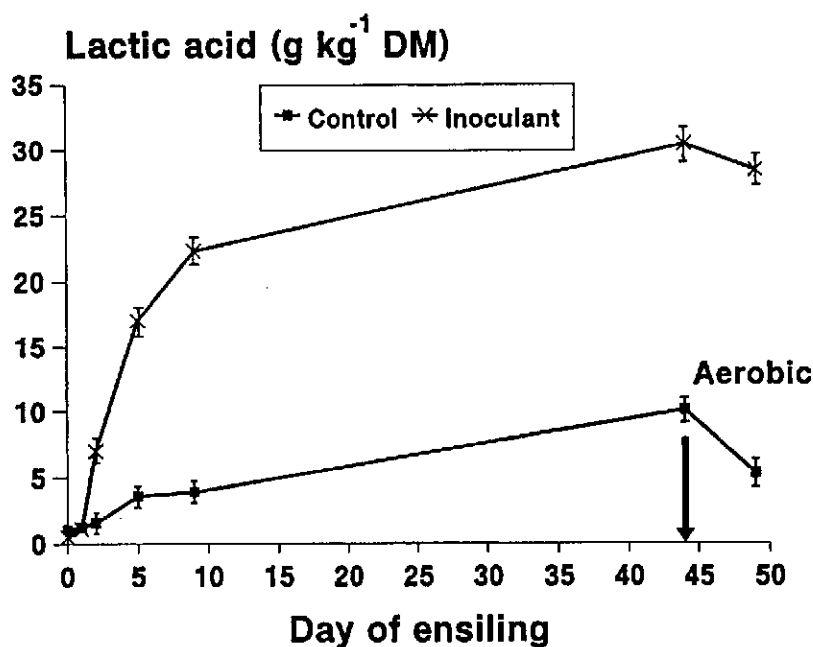


Figure 2. The change in lactic acid of *D. eriantha* silage ensiled with or without a lactic acid bacterial inoculant. SEM = 0.24 (Standard error bars are shown where significant differences ($p < 0.05$) were found).

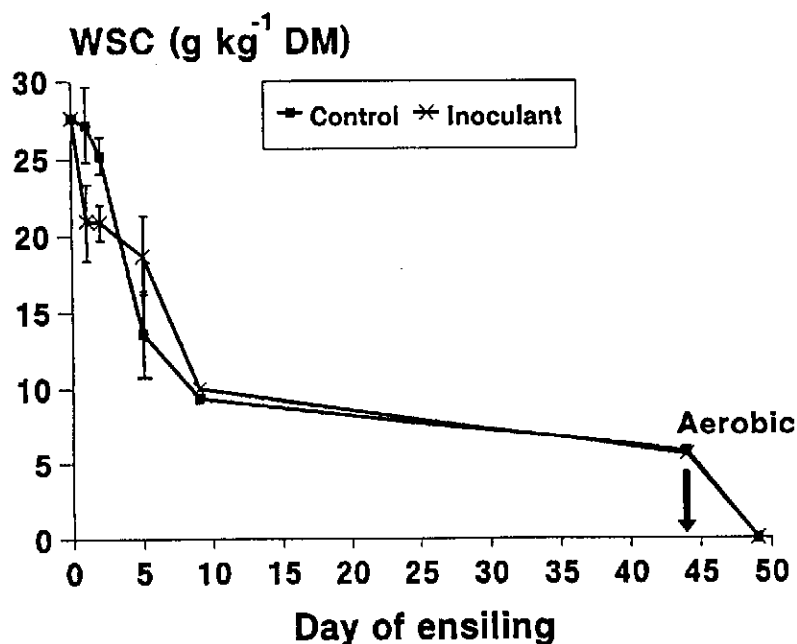


Figure 3. The change in water soluble carbohydrate of *D. eriantha* silage ensiled with or without a lactic acid bacterial inoculant. SEM = 0.35 (Standard error bars are shown where significant differences ($p < 0.05$) were found).

Table 1

Composition (g kg⁻¹ DM) of *D. eriantha* silage at 44 days after ensiling, made with or without the addition of a lactic acid bacterial inoculant (Laboratory study, $n = 3$)

	Control	Inoculant	SEM ^g
Dry matter	383 ^a	369 ^b	2.7
Organic matter	905 ^a	907 ^b	0.6
NDF	724	716	4.9
IVOMD ^c	520	534	17.9
Crude protein	65	61	2.6
NH ₃ -N g kg ⁻¹ TN ^d	50.3	35.4	9.9
pH	4.87 ^a	3.90 ^b	0.10
WSC ^e	6	8	1.5
Lactic acid	10 ^a	31 ^b	1.4
Acetic acid	9.9	6.4	1.4
Butyric acid	ND ^f	ND	
Propionic acid	0	0.2	0.1

^{a,b}Means with different letters in the same row differ significantly ($p < 0.05$).

^c*In vitro* organic matter digestibility, ^dTotal nitrogen, ^eWater soluble carbohydrate,

^fNot detected, ^gStandard error of means.

3.2. Intake and digestibility study

The chemical composition of silage made in the tower silos and that of the hay is given in Table

3. The chemical composition of the three diets fed to Merino rams is given in Table 4.

The intake and *in vivo* organic matter digestibility of the different diets is given in Table 5. The DM intake of control and inoculated silage diet was 10% and 32% higher respectively than that of the hay diet.

The *in vivo* organic matter digestibility of the inoculated silage diet was significantly ($p < 0.05$) higher than that of the control silage.

Table 2

Microbial examination (log CFU g⁻¹ fresh material) of *D. eriantha* ensiled with or without a lactic acid bacterial inoculant.

Treatment	Day of ensiling	Lab ^a	E ^b	Y ^c	M ^d	CL ^e
Control						
Fresh material	0	1.6	5.2	6.5	4.8	0.7
Silage	1	5.8	7.1	5.1	4.3	0.5
	2	6.4	5.7	5.1	4.1	0.7
	5	7.1	4.1	5.7	4.0	1.05
	9	7.5	6.3	4.2	3.2	1.9
	44	7.2	NF ^f	3.2	2.0	2.0
Aerobic	5	8.1	NF	7.8	6.0	1.0
Inoculant						
Fresh material	0	3.7	4.3	5.1	4.0	1.1
Silage	1	7.7	6.1	5.0	3.2	0.4
	2	8.0	5.1	4.1	3.0	0.6
	5	8.2	2.7	4.0	2.9	0.2
	9	8.1	4.0	4.8	NF	0.4
	44	6.0	NF	2.7	NF	0.6
Aerobic	5	7.4	NF	8.0	NF	2.0

^aLactic acid bacteria, ^b Enterobacteria, ^cYeast, ^d Mould, ^e Clostridial spores, ^f Not found.

Table 3

The chemical composition (g kg^{-1} DM) of *D. eriantha* silage made in tower silos with or without the addition of a lactic acid bacterial inoculant and that of hay. (The standard deviation (\pm) is given after the mean ($n = 4$).)

	Silage		Hay
	control	inoculant	
Dry matter	382 ± 12	432 ± 14	915 ± 8
Organic matter	905 ± 12	917 ± 12	916 ± 11
NDF ^a	713 ± 14	716 ± 16	717 ± 15
IVOMD ^b	570 ± 17	583 ± 14	563 ± 13
Crude protein	61 ± 12	59 ± 14	51 ± 15
NH ₃ -N (g kg^{-1} TN ^c)	80 ± 12	36 ± 5	
pH	4.63 ± 0.3	4.14 ± 0.1	
WSC ^d	16.5 ± 1.1	22.0 ± 1.5	
Lactic acid	21.7 ± 1.3	28.6 ± 2.1	
Acetic acid	15.2 ± 0.6	5.9 ± 0.8	
Butyric acid	7.5 ± 3.2	1.6 ± 0.9	
Propionic acid	0.5 ± 0.2	0	

^aNeutral detergent fibre, ^b*In vitro* organic matter digestibility, ^cTotal nitrogen.

^dWater soluble carbohydrate.

Table 4

The chemical composition (g kg^{-1} DM) of diets containing 90.9% *D. eriantha* silage or hay fed to Merino rams. (The standard deviation (\pm) is given after the mean ($n = 4$).)

	Silage		Hay
	control	inoculant	
Dry matter (g kg^{-1} Diet)	385 ± 6	425 ± 15	900 ± 0.9
Organic matter	880 ± 1	887 ± 14	906 ± 6
NDF ^a	641 ± 12	624 ± 10	674 ± 13
IVOMD ^b	597 ± 11	634 ± 12	552 ± 17
Crude protein	103 ± 5	102 ± 7	96 ± 4

^aNeutral detergent fibre, ^b*In vitro* organic matter digestibility.

Table 5

In vivo organic matter digestibility and intake of *D. eriantha* silage and hay diets fed to mature Merino rams ($n = 8$ per treatment)

	Silage		Hay	SEM ^f
	control	inoculant		
Digestibility (g kg ⁻¹ DM)				
organic matter	546 ^a	574 ^b	561 ^b	7
Intake				
g DM day ⁻¹	1540 ^b	1848 ^c	1395 ^a	56
g DOMI ^d kg ⁻¹ W ^{0.75} day ⁻¹	38.1 ^b	42.6 ^c	32.1 ^a	1.1
DMI ^e g kg ⁻¹ liveweight	25 ^b	30 ^c	22 ^a	1

^{a,b,c} Means with different letters in the same row differ significantly ($p < 0.05$).

^d Digestible organic matter intake, ^e Dry matter intake, ^f Standard error of means.

4. Discussion

4.1. Laboratory study

The addition of the lactic acid bacterial inoculant and enzymes caused higher levels of LA (Fig. 2) which resulted in a more rapid drop in pH (Fig. 1) compared to the control silage. Meeske and Basson (1998a) found similar results when adding a LAB inoculant to *E. curvula* (subtropical grass). The WSC content of subtropical grasses is generally lower than that of temperate species (McDonald *et al.*, 1991). The low WSC content of *D. eriantha* at ensiling and lower level of lactic acid bacteria (Table 2) may have contributed to the very slow rate in pH drop of the control silage (Fig. 1). Meeske and Basson (1998b) found that the WSC content of maize at ensiling was 107 g kg⁻¹ DM and the pH dropped to four after two days of ensiling. After two days of ensiling the pH of the inoculated *D. eriantha* silage was still 5.28. The low amount of available WSC may have restricted the growth of lactic acid bacteria, preventing a faster drop in pH in the inoculated silage. It has been shown that grasses of tropical and subtropical origin accumulate starches composed of amylose and amylopectin instead of fructans in their vegetative tissues (Smith, 1973). Therefore, the amylase added to the inoculant treatment could have increased the available

WSC to the lactic acid bacteria in the inoculated silage. This theory is supported by the reduced drop in WSC of the inoculated silage from day 2 to day 5 of ensiling (Fig. 3). The WSC content of the control and inoculated silage did not differ significantly after day 9 of ensiling (Fig. 3).

After nine days of ensiling the pH of the control and the inoculated silage was 5.73 and 4.14, respectively (Fig. 1). The slower rate of pH drop in the control silage allowed more time for the growth of clostridia as indicated by the higher number of clostridial spores (Table 2) compared to the inoculated silage. The growth of most acid-tolerant clostridia is inhibited by a pH just below 5 (Jonsson, 1991).

The counts of LAB were higher on the inoculant treated silage compared to the control silage (Table 2). Although the number of CFU of LAB/g fresh material of the treated silage on day 0 is more than 100 times higher than that of the control, it is much lower than expected. The use of a low pH Rogosa agar as incubation media to enumerate LAB may have resulted in an underestimation of LAB numbers (Rooke, 1990). Pitt and Leibensperger (1987) summarised the literature concerning the numbers of epiphytic LAB found on forage crops after harvesting by conventional farm machinery and found a mean value of $3.7 \log_{10}$ CFU g^{-1} herbage. In the present study, only $1.6 \log_{10}$ CFU LAB g^{-1} herbage was found on the control treatment prior to ensiling. Dry, hot conditions may have had a negative effect on the numbers of epiphytic LAB. The counts of enterobacteria, yeast and mould (Table 2) were lower on the inoculated silage compared to that of the control silage. This could have been caused by the higher levels of lactic acid in the inoculated silage which would have inhibited yeasts growth (McDonald *et al.*, 1991). No mould was detected in the inoculated silage after day 5 of ensiling when the pH was 4.4. When exposed to air the rapid increase in pH of the control silage compared to the inoculated silage can be partly explained by the high mould counts (10^6 CFU of mould per gram of silage) on the control silage while no mould was detected on the inoculated silage. Most yeasts will grow within the pH range 3-8 and some strains are able to withstand acidities of pH 2 or below (McDonald *et al.*, 1991). When the silage was exposed to air the yeast counts of control and inoculated silage increased drastically.

When the silage was exposed to air for five days, the pH increased from 4.87 to 6.75 in the control silage and from 3.9 to 4.51 in the inoculated silage (Fig. 1). The inoculated silage was more stable than the control silage when exposed to air, as indicated by the lower production of CO₂, slower increase in pH and no mould detected in the inoculated silage compared to the control silage. Improved aerobic stability of silage can be expected when lactic acid bacteria are added and the pH is lowered quickly (McDonald *et al.*, 1991). The aerobic stability of silage may be reduced by the addition of bacterial inoculants, as found by Kung *et al.* (1991) and Meeske *et al.* (1993). This may occur when the silage contains a high level of water soluble carbohydrates (60 g WSC kg⁻¹ DM) and lactic acid (70 g LA kg⁻¹ DM) in the presence of high numbers of lactate assimilating yeasts (Meeske *et al.*, 1993).

4.2. Intake and digestibility study

The addition of the inoculant to *D. eriantha* silage made in tower silos resulted in silage with a lower pH, less proteolysis (lower level of ammonia-N as percentage of total nitrogen), lower acetic and butyric acid content and a higher lactic acid content (Table 3) compared to control silage. According to McDonald *et al.* (1991) these differences in silage composition, as well as the higher *in vivo* organic matter digestibility, may explain the significantly ($P < 0.05$) higher intake of the inoculated silage diet compared to the control silage diet (Table 5). The intake of inoculated silage diet at 30 g DM kg⁻¹ of liveweight was 20% higher than that of the control silage diet. The intake of the untreated silage diet by Merino rams at 25 g DM kg⁻¹ of liveweight in the present study compares well with the intake of 22 g DM kg⁻¹ of liveweight found by Hardy *et al.* (1990) when untreated *D. eriantha* silage was fed to Hereford cows.

The lower *in vivo* organic matter digestibility of the control silage diet, compared to the inoculated silage diet, may be partly explained by the higher proteolysis in the former. Aerobic stability was not determined on the silage made in the tower silos, but it can be expected that the control silage was less stable than inoculated silage as was found in the laboratory study.

The intake of both silage diets by Merino rams was higher than the intake of the hay diet. This

agrees with the findings of Petit *et al.* (1985) but disagrees with the findings of Petit and Flipot (1992), Thiago *et al.* (1992) and Campling (1966) who found a lower intake of silage compared to that of hay. The intake of silage is quite variable and depends on fermentation characteristics (Demarquilly, 1973). The milling of the hay through a hammermill increased the dust and this may have contributed to the lower intake of the hay diet. Cushnahan and Gordon (1995) also found lower intakes of hay compared to silage. They attributed this to difficult hay making conditions.

5. Conclusions

The use of a lactic acid bacterial inoculant with enzymes when ensiling *D. eriantha*, resulted in an improved preservation as indicated by a more rapid drop in pH, higher levels of lactic acid, lower numbers of enterobacteria, yeast and mould and an improvement in the aerobic stability of the silage. The intake and *in vivo* organic matter digestibility of *D. eriantha* silage were significantly improved by the use of the inoculant.

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Chapter 3

The ensiling of lucerne

The effect of a lactic acid bacterial inoculant and molasses on the fermentation dynamics of lucerne silage

The effect of a lactic acid bacterial inoculant and type of baler on the composition of big bale lucerne silage

1. Introduction

Lucerne can be preserved as hay or silage. Nelson and Satter (1992) found that cows fed lucerne silage consumed 1.2 kg more dry matter and produced 2.1 kg more milk per day than cows fed lucerne hay. Broderick (1995) and Beauchemin *et al.* (1997) however found no effect on milk production when feeding lucerne preserved as hay or wilted silage (DM 340 to 400g kg⁻¹) to dairy cows.

Lucerne is not an easy crop to ensile because of its low water soluble carbohydrate and low dry matter content, high protein content and high buffering capacity. This means that it resists pH change and that nutrients available to the lactic acid bacteria to produce lactic acid may be limiting. (McDonald *et al.*, 1991). The efficient use of the limited amount of water soluble carbohydrates present in lucerne by the addition of a homofermentative lactic acid bacterial inoculant at ensiling, should be beneficial to preservation. Insufficient numbers of viable lactic acid bacteria on a crop at ensiling will cause a delay in the drop of pH, a higher nutrient loss and silage of a poorer palatability (Woolford 1984).

Homofermentative lactic acid bacteria can be added at ensiling to ensure that sufficient viable lactic acid bacteria are present (Woolford 1984). Studies on the addition of inoculants to lucerne have shown positive effects (Seale *et al.*, 1986; Weinberg *et al.*, 1988; Rooke and Kafizadeh, 1994) and no effect (Ohyama *et al.*, 1973; Ely *et al.*, 1982; Lindgren *et al.*, 1983). The efficiency of lactic acid bacterial inoculants is determined by the species and strains of lactic acid bacteria that are selected (Moon, 1981). Satter *et al.* (1987) showed that only when the inoculant supplied 10 times as many lactic acid bacteria as were on the lucerne prior to ensiling, could an animal response be shown. The number of epiphytic lactic acid bacteria present on lucerne prior to ensiling is determined by forage harvester inoculation, average wilting temperature in the swath, wilting time and the rate of drying (Muck, 1989). Oshima *et al.* (1997) showed that the quality of lucerne silage was poor when unwilted lucerne was ensiled without an additive at a dry matter content of 180 g kg⁻¹ fresh material. Wilting of legumes is often recommended as this will concentrate the available water soluble carbohydrates (McDonald *et al.*, 1991) and will increase

the number of lactic acid bacteria (Muck, 1989).

Phillip *et al.* (1990) wilted lucerne to 500g DM kg⁻¹ silage and found that the addition of a bacterial inoculant to silage altered the fermentation slightly during the first 7 days of ensiling but compared to untreated silage, compositional differences were minimized as fermentation extended beyond 42 days. Inoculation of lucerne did not affect voluntary intake of silage by sheep. Fredeen *et al.* (1991) found that composition of inoculated lucerne silage did not differ from that of untreated lucerne silage and that cow performance was not affected the adding of the inoculant.

Big bale silage is becoming more popular in South Africa as is also the case in Europe. The average ambient temperature in South Africa is higher than in Europe and this increases the risk of surface moulding on big round bales (Randby, 1996). The use of an inoculant when making big bale lucerne silage has not been investigated in South Africa.

The aim of this study was to determine the effect of the addition of a lactic acid bacterial inoculant or molasses to lucerne at ensiling on the fermentation dynamics, the composition and aerobic stability of lucerne silage. In the second part, the effect of a lactic acid bacterial inoculant and type of baler on the composition of big bale lucerne silage was determined.

2. Materials and methods

2.1. Laboratory study

Lucerne (*Medicago sativa*) cultivar CUF 101 was cut at 15% flowering stage on the 19th of April 1994 at Roodeplaat Grassland Institute (longitude 28° 21'S : latitude 25° 35'E, altitude 1504m) between 8:00 and 08:30. The lucerne was wilted for four hours and chopped with a Feraboli 945 precision silage chopper. Sixty kilogram of chopped lucerne was thoroughly mixed and divided in three portions of twenty kilograms, for the control, inoculant and molasses treatments. The lucerne was ensiled with or without the addition of a lactic acid bacterial (LAB) inoculant or molasses in 1.5 litre glass jars, equipped with a special lid with springs which enables gas release

only (J. WECK, GmbH u. Co., Wehr-Oflingen, W. Germany). The LAB inoculant (Sil-All) contained *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with enzymes amylase and cellulase. The inoculant was applied at 10 g per tonne of fresh material to provide 10^6 colony forming units (CFU) of lactic acid bacteria per gram of material. Molasses was applied at two percent of fresh material. Representative samples of fresh chopped lucerne were taken and frozen at -20°C .

Eighteen 1.5 litre mini silos were filled for each treatment to follow the fermentation dynamics during the ensiling period on laboratory scale. Three silos were be opened for each treatment on each of day 1, 2, 4, 7, 14 and 90 after ensiling. Representative samples of silage from these jars were taken for DM determination and chemical analysis (stored at -20°C). At day 90 of ensiling, silage was exposed to air for 5 days to determine the aerobic stability according to the method of Ashbell *et al.* (1991). According to this method CO_2 production during the aerobic deterioration is measured as an indicator of aerobic spoilage of silage.

Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 hours. Dried silage was ground with a Wiley laboratory mill through a 1mm sieve. *In vitro* organic matter digestibility (IVOMD) of dried silage was determined according to Tilley and Terry (1963) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Total nitrogen was determined by the Kjeldahl method. Water soluble carbohydrate (WSC), pH and lactic acid were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 minutes with a stomacher. Water soluble carbohydrates were determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956) and lactic acid was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acid (VFA) content of silage was determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35m x 3mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H_3PO_4). The column was conditioned for 48 hours at 165°C with a nitrogen carrier gas flow of 40 ml per minute. The ammonia nitrogen content of silage was determined by homogenizing 50g of silage in 250ml of a 0.1N H_2SO_4 solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia

content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Mettler 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen. Least significant differences between treatments were determined using Statgraphics (1988).

2.2. Big bale lucerne silage

Lucerne was cut at the 10% flowering stage at Roodeplaat Grassland Institute (longitude 28° 21'S : latitude 25° 35'E, altitude 1504m) at 8H30 and was wilted for four hours. The lucerne was raked in four wind rows and two rows were baled with either a fixed press chamber big round baler (Welger RP200) or a baler with a variable press chamber (New Holland 640). Inoculant was applied (10 g tonne⁻¹ fresh material, 2 litre per tonne) to one wind row for each baler by spraying the inoculant at the pick up of the baler. A representative sample of lucerne was collected in the wind row for each bale just prior to baling. Samples were stored at - 20°C. Eight bales were made with each baler of which four were treated with a bacterial inoculant. The bales were wrapped (four layers of plastic) with a big bale wrapper. Bales were transported to the Irene Animal Production Institute and were stored outside without provision of shade. The volume of each bale was determined and each bale was weighed on day 0 and day 120 of ensiling. After 120 days of ensiling representative samples of each bale were taken with a silage corer and DM, OM, nitrogen content, ammonia, IVOMD, WSC, lactic acid, VFA content were determined as described for the laboratory study. Data was processed by one way analysis of variance determining the least significant differences using Statgraphics (1988).

3. Results and discussion

3.1. Laboratory study

The adding of the inoculant resulted in a rapid drop in pH from 6.25 at ensiling to 4.5 after 4 days of ensiling as shown in Figure 1. After 7 days of ensiling the pH of the control, inoculated and molasses treated lucerne silage differed significantly ($P < 0.05$) and was 5.07^a, 4.5^c and 4.7^b

respectively. The rapid drop in pH on the inoculant and molasses treatment was caused by higher lactic acid production. The lactic acid content (% of DM) of control, inoculated and molasses treated lucerne silage was 0.32^a, 4.54^b and 0.43^a after two days, 1.88^a, 5.34^c and 2.55^b after four days and 3.85^a, 6.04^c and 5.10^b after seven days of ensiling respectively (Figure 2).

The changes in WSC that occurred during the ensiling period in the lucerne silages are presented in Figure 3 . The adding of molasses did result in a significantly ($P<0.05$) higher WSC content of lucerne silage on day 90 of ensiling compared to that of the control and inoculated lucerne silage. This higher WSC content did not have a detrimental effect on the aerobic stability of the lucerne silage. The WSC content of lucerne prior to ensiling was 4.7% of DM which was lower compared to the 7.2% (Weinberg *et al.*, 1988), 10% at the bud stage (Kung, Jr., *et al.*, 1984), 9.2% at the bud stage (Kent, *et al.*, 1989), 7.7% at 10% bloom stage (Kung *et al.*, 1991) reported by others. After 90 days of ensiling, the inoculated and control lucerne silage contained only 0.9% and 0.7% WSC in our study, which is very low compared to the 3%, 2%, 3% and 3.4% WSC reported by the respective authors on lucerne silage.

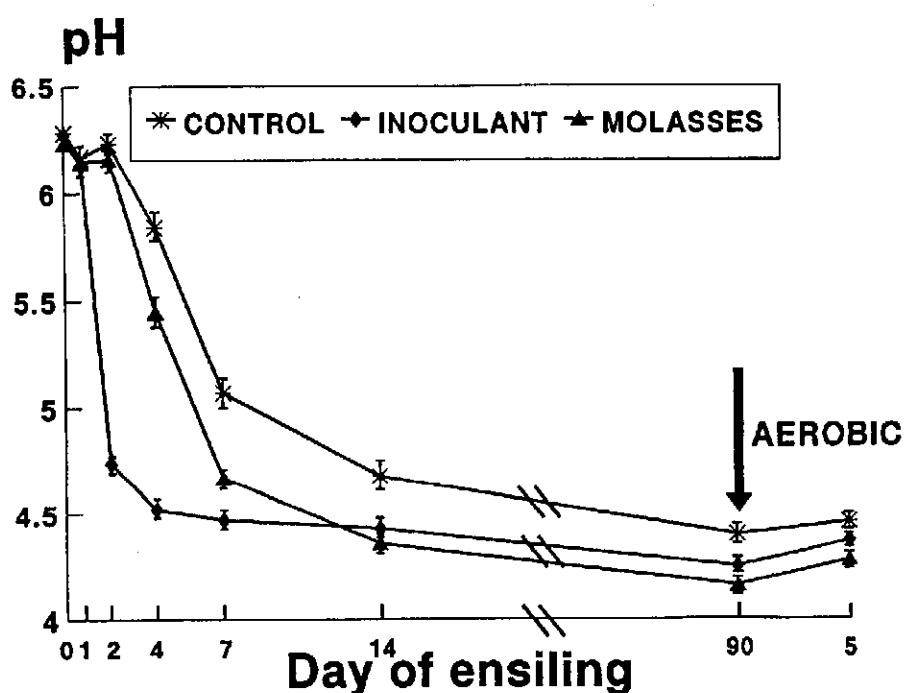


Figure 1. The change in pH of lucerne ensiled with or without a lactic acid bacterial inoculant or molasses. (Standard error bars are shown where significant ($P<0.05$) differences were found)

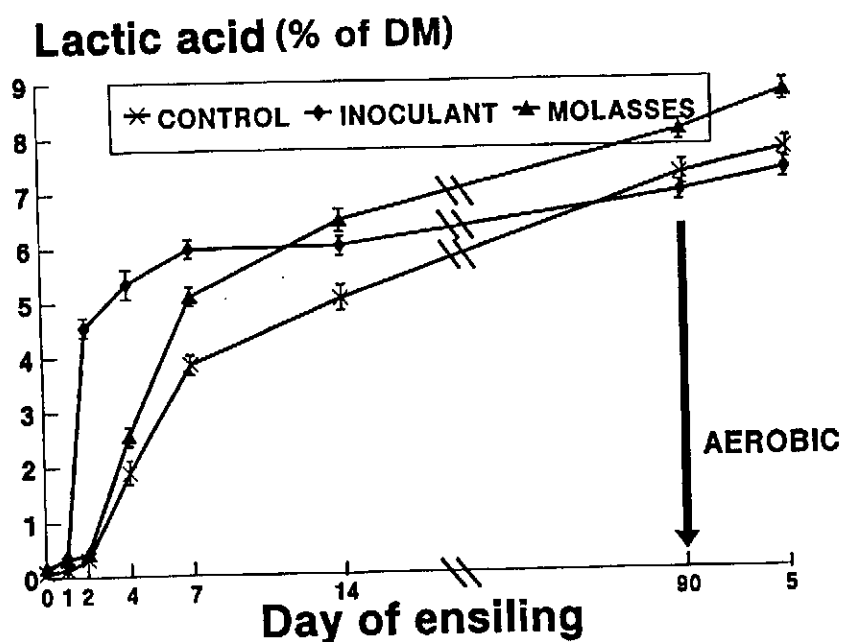


Figure 2. The change in lactic acid of lucerne ensiled with or without a lactic acid bacterial inoculant or molasses. (Standard error bars are shown where significant ($P < 0.05$) differences were found)

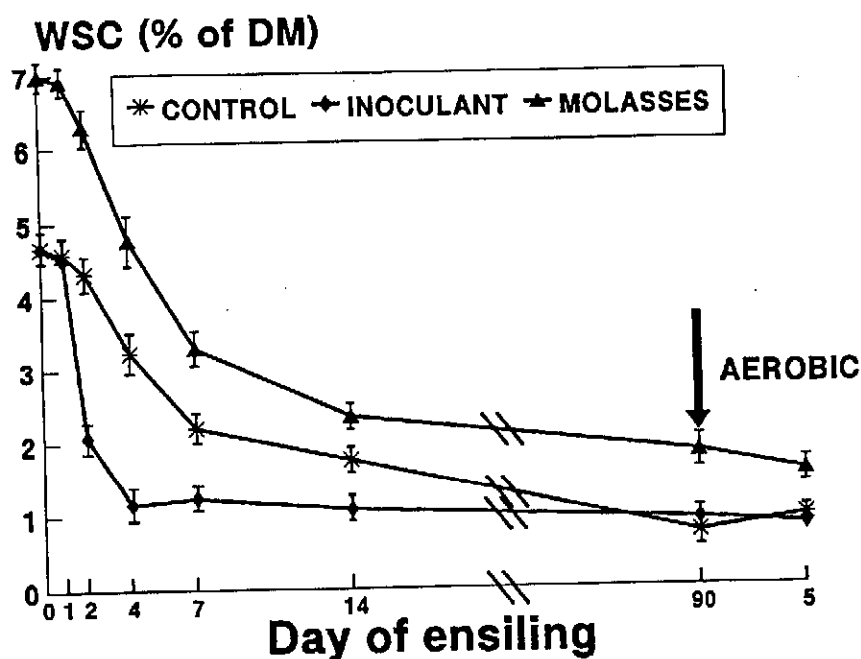


Figure 3. The change in water soluble carbohydrates of lucerne ensiled with or without a lactic acid bacterial inoculant or molasses. (Standard error bars are shown where significant ($P < 0.05$) differences were found)

The stage at which lucerne is harvested effects its WSC content. The highest levels WSC can be expected at the pre-bud growth stage (McDonald *et al.*, 1991). Although the amount of WSC utilized during the ensiling of the untreated and inoculated lucerne silage were similar, the rate of utilization was faster in the inoculated lucerne silage (Figure 3).

The composition of the silages after 90 days of ensiling is presented in Table 1.

Table 1.

Composition (% of DM) of lucerne silage made with and without adding of a lactic acid bacterial inoculant or molasses.

	Control	Inoculant	Molasses	SEM ^d
Dry matter	35.6 ^a	35.8 ^a	37.1 ^b	.10
Organic matter	90.2	90.1	90.0	.19
NDF	47.6 ^{ab}	48.2 ^a	46.6 ^b	.30
IVOMD	63.9	63.5	64.9	.90
Crude protein	19.7	20.1	19.5	.38
NH ₃ -N (% of TN)	8.0 ^a	5.9 ^b	6.8 ^b	.28
pH	4.4 ^a	4.3 ^b	4.2 ^c	.01
WSC ^e	0.7 ^a	0.9 ^a	1.9 ^b	.08
Lactic acid	7.3 ^a	6.9 ^a	8.1 ^b	.17
Acetic acid	3.9 ^a	2.7 ^b	3.5 ^a	.18
N-Butyric acid	NF ^f	NF	NF	
Iso-Butyric acid	NF	NF	NF	
Propionic acid	NF	NF	NF	
Isovaleric acid	0.13	0.17	0.14	.18
n-Valeric acid	1.42	0.88	1.31	.27

^{a,b,c} Means with different superscripts in the same row differ significantly ($P < 0.05$),

^d SEM = Standard error of means, ^e WSC = Water soluble carbohydrates, ^f NF = Not found

Both the inoculant and the molasses resulted in less ($P < 0.05$) break down of protein and a lower pH compared to that of the control silage. No butyric acid was found in any of the silages. The lower acetic acid content of the inoculated silage compared to the control and molasses treated

silage indicates that the homofermentative lactic acid bacteria did grow effectively. Jones *et al.* (1992) showed that as level of inoculation was increased, fermentation was more homofermentative and lower levels of acetic acid were found in lucerne silages. The level of acetic and lactic acid found in our study compares well with that reported by (Jones *et al.*, 1992; Dodo and Allen, 1996).

All the silages were stable when exposed to air for 5 days. The low pH, substantial amount of acetic acid and low level of water soluble carbohydrates may have contributed to the aerobic stability of the silages. This finding agrees with that of Lindgren *et al.* (1985). Kung *et al.* (1991) however, found that lucerne silages treated with inoculant were less stable when exposed to air than untreated control silage. The acetic acid content of inoculated lucerne silage was however lower (1.9% of DM) and the WSC higher (3.4% of DM) in their study, compared to the 2.7 to 3.5% acetic acid and 0.7 to 0.9 % WSC found in our study. Muck and O'Keily (1992) studied the aerobic deterioration of lucerne silages and found that all laboratory scale lucerne silages were stable while three out of ten farm-scale lucerne silages were unstable when exposed to air. Higher level volatile fatty acids like propionic acid were not particularly low in unstable silages compared with the others. Unstable silages were however lower in 2,3-butanediol than other silages. This is a compound that is produced by lactic acid bacteria, enterobacteria and bacilli.

3.2. Big round bale silage

The composition of big round bale lucerne silage is given in Table 2. The inoculated silages had a lower pH, butyric acid and ammonia-nitrogen content and higher levels of lactic acid than control silages. Similar results have been found by Kung *et al.* (1991) when wilted lucerne was ensiled with an microbial inoculant. The amount of total nitrogen present as ammonia was very high on the control silages (Table 3) indicating poor fermentation. Lancaster *et al.* (1977) found that untreated lucerne silage with a pH of 5.22, a dry matter content of 230 g kg⁻¹ and ammonia nitrogen content of 242 g NH₃-N kg⁻¹ total nitrogen was unpalatable. This resulted in poor intakes and animal performance.

The pH of control silage made with the variable baling chamber (VBC) baler did not differ significantly ($P < 0.05$) from that of silage made with the fixed baling chamber (FBC) baler with the addition of inoculant. Bales made with the VBC baler were significantly ($P < 0.05$) heavier (677kg) than bales made with the FBC baler (593kg). The bale density was 141 and 153 kg DM m^{-3} for bales made with the FBC and VBC baler respectively. The amount of silage discarded due to moulding and poor fermentation was 111 and 71 kg/tonne ($P = 0.12$) for bales made with the FBC and the VBC baler respectively.

Table 2.

The composition (% of DM) of big bale lucerne silage made with a fixed or a variable bale chamber baler with or without the addition of an inoculant.

	Type of baler				
	Fixed chamber		Variable chamber		SEM ^c
	Control	Inoculant	Control	Inoculant	
Dry matter	25.1 ^a	29.4 ^b	31.1 ^b	29.6 ^b	1.2
Organic matter	85.9 ^a	87.3 ^b	87.5 ^b	86.2 ^a	0.25
IVOMD ^d	62.9 ^a	65.3 ^a	65.5 ^{ab}	66.9 ^b	1.2
N	3.26 ^a	3.29 ^a	3.24 ^a	3.69 ^b	1.7
NH ₃ -N (% of TN ^e)	18.8 ^a	13.5 ^b	15.3 ^{ab}	11.1 ^b	14
pH	5.12 ^a	4.82 ^b	4.99 ^{ab}	4.75 ^b	0.09
WSC ^f	1.03	1.19	1.32	1.14	0.13
Lactic acid	3.08 ^a	4.17 ^b	2.57 ^a	4.35 ^b	0.35
Acetic acid	5.06	5.07	3.58	5.0	0.62
N-Butyric acid	0.28 ^a	0.004 ^b	0.04 ^b	0.001 ^b	0.03
Iso-Butyric acid	0.01 ^a	0.005 ^{ab}	0.003 ^{ab}	0 ^b	0.003
Propionic acid	0.14 ^a	0.03 ^b	0.06 ^b	0.03 ^b	0.02
Isovaleric acid	0.05 ^a	0.04 ^{ab}	0.04 ^{ab}	0.03 ^b	0.005
n-Valeric acid	0.02 ^a	0.07 ^b	0.03 ^a	0.08 ^b	0.011

^{a,b} Means with different superscripts in the same row differ significantly ($P < 0.05$)

^c SEM= Standard error of means, ^dIVOMD= *In vitro* organic matter digestibility,

^eTN= Total nitrogen, ^fWSC= Water soluble carbohydrates

The adding of an inoculant to big round bale lucerne silage made with a fixed baling chamber baler, resulted in an improved preservation compared to the untreated control silage as was indicated by a lower pH, higher lactic acid content, lower ammonia nitrogen and lower butyric acid content.

The effect of inoculation was however less pronounced in big bale lucerne silage made with a variable baling chamber baler. Here inoculation did result in a higher lactic acid content compared to the untreated control lucerne silage.

The acetic acid content of all big bale lucerne silages were higher than the 2.87% of DM found by Luchini *et al.* (1997) in bunker lucerne silages. The lactic acid content of the big bale lucerne silages was lower than that found in lucerne ensiled in laboratory silos but is similar to that found by Luchini *et al.* (1997) in bunker lucerne silages.

The composition of silage made with the two different balers did not differ significantly. This can be explained by the fact that bale density did not differ significantly ($P < 0.05$) between bales made with the different balers. Silage losses due to moulding and poor fermentation tended to be higher with the fixed baling chamber baler compared to the variable baling chamber baler.

4. Conclusions

The addition of an inoculant and addition of molasses to lucerne ensiled in laboratory silos resulted in an increased preservation rate as indicated by a more rapid lowering of pH and a faster rate of lactic acid production and less protein breakdown compared to control silage. The inoculant was more effective than the molasses in improving the rate of preservation. The aerobic stability of lucerne silage was not affected by inoculation or the addition of molasses. The amount of water soluble carbohydrates present in lucerne prior to ensiling was 3 to 5% lower than that reported by other authors. The addition of an inoculant to wilted big bale lucerne silage did improve silage quality as indicated by a lower pH, higher lactic acid content, lower ammonia nitrogen content and lower level of butyric acid. Big round bale lucerne silage differed markedly

from lucerne ensiled in laboratory silos as the former had a higher pH, ammonia nitrogen, butyric acid and acetic acid content and a lower lactic content.

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Chapter 4

The ensiling of forage sorghum.

Ensiling forage sorghum at two stages of maturity with the addition
of lactic acid bacterial inoculants

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Introduction

Sorghum should be used more extensively as silage crop in South Africa. Sorghum is more drought tolerant than maize (Beadle *et al.*, 1973) and has an extended planting period which makes it a useful crop in areas of low or uncertain rainfall (McDonald *et al.*, 1991). Meeske and Basson (1995) found that sorghum had a higher yield than maize under drought conditions and that drought stricken sorghum could be ensiled without any additives.

The digestibility of sorghum is at its best 80 to 85% of that of maize (McDonald *et al.*, 1991). Sorghum tends to have a higher sugar content and a lower starch content than maize prior to ensiling (McDonald *et al.*, 1991). Ward *et al.* (1966) showed that intake of sorghum silage was almost perfectly correlated ($r=0.98$) with dry matter percentage and recommended that sorghum should be ensiled at the greatest practical dry matter content. There is a positive correlation between the grain content of sorghum and its digestibility (White *et al.*, 1991).

Adding an inoculant to sorghum at ensiling may improve the fermentation dynamics during ensiling. Several trails have shown that calves fed on inoculated sorghum silages had improved daily gains and feed efficiencies compared with those fed on control silages (Bolsen and Ilg, 1981). Hinds *et al.* (1985) found that adding an inoculant to sorghum did not improve daily gain or feed conversion rate of steers. The inoculated silage did however loose less DM during fermentation, storage and feed out than the control.

The aim of the following paper was to determine the effect of lactic acid bacterial inoculants on the composition, fermentation dynamics during ensiling and aerobic stability of forage sorghum silage made at two stages of maturity.

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Ensiling forage sorghum at two stages of maturity with the addition of lactic acid bacterial inoculants

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Abstract

Whole crop forage sorghum cultivar FS2 was harvested at the late bloom (20.7% dry matter (DM)) and soft dough (28.9% DM) stages of maturity. The sorghum was chopped to approximately 10 mm pieces and ensiled under laboratory conditions in 1.5 l Weck glass jars. At ensiling, it was treated with two commercial silage inoculants: H/M F and Sil-All. The inoculants were applied at 10^6 colony-forming units g^{-1} of fresh material. Silage with no additives served as a control. Three jars per treatment were opened on Days 1, 2, 5, 10 and 31 post-ensiling to study fermentation dynamics. After 31 days of ensiling the silages were analysed and subjected to an aerobic stability test lasting 5 days. The yield of the sorghum harvested at the late bloom and the soft dough stages was 11.8 ton DM ha^{-1} and 16.4 ton DM ha^{-1} , respectively. The IVOMD was 61.4% and 67.6%, respectively. At both stages of maturity the inoculants caused a more rapid rate of pH decline and a higher amount of lactic acid production. All the silages were well preserved. Silages of the sorghum ensiled at the late bloom stage with all treatments were stable after 5 days of aerobic exposure, whereas sorghum ensiled at the soft dough stage with the addition of the inoculants deteriorated upon aerobic exposure. This was evident from a significantly ($P < 0.05$) higher production of CO_2 as compared with the control, and increase in pH. It is concluded that addition of lactic acid bacterial inoculants to mature sorghum at ensiling might impair the aerobic stability of the silage.

1. Introduction

The main summer forage crop for silage in Israel, South Africa and many other areas is corn, because of its high dry matter yields, excellent ensiling characteristics and high digestibility. However, corn requires intensive irrigation, and because of the chronic water shortage in Israel and dry summers in South Africa, forage sorghum is becoming more important as a fodder crop in these countries, owing to its high yields and drought tolerance.

The nutritional value of forage sorghum generally declines with increasing maturity. However, in high grain producing sorghum, the digestible starch fraction in the kernel may result in improved nutritional value as the plant matures. Variations in nutrient digestibility exist between and within cultivars (Dickerson, 1986). Decreased digestibility in mature forage sorghum is attributed to increased lignification and the formation of lignin-hemicellulose complexes (Goto *et al.*, 1991). Various studies have indicated the decline in digestibility of forage sorghum with maturation (e.g. Fox *et al.*, 1970; Black *et al.*, 1980; Dannhauser *et al.*, 1990). However, there is no conclusive evidence on the effect of age at harvest in high grain producing forage sorghum cultivars on the nutritive value.

Forage sorghum is usually conserved by ensiling at the late milk to soft dough stage of maturity, when the plants are fully grown and have a dry matter (DM) content of 27-30% (Black *et al.*, 1980). Dickerson (1986) ensiled various sorghum cultivars with DM content between 24 and 42% and obtained well-preserved silages, with low pH values and high content of lactic acid. The lower end of the DM content might be somewhat too low for adequate lactic acid fermentation (Woolford, 1984). One possible approach to improve the ensiling fermentation of moist crops, which is increasing in popularity, is to add homofermentative lactic acid bacteria (LAB) inoculants at ensiling (e.g. Weinberg *et al.*, 1988, 1993). The benefits attributed to such inoculants are fast and intensive production of lactic acid, and a rapid decline in pH, which stabilizes the silage. The purpose of the experiment described here was to study the effect of stage of maturity of a high grain producing forage sorghum on quality and ensiling characteristics with or without the addition of LAB inoculants.

2. Materials and methods

2.1 Experimental

Forage sorghum cultivar FS2 (Dekalb, Plant Genetics, Labbock, TX) was harvested at the late bloom and soft dough stages of maturity. The whole plants were chopped into pieces of about 10 mm using a laboratory chopper (Winterschteiger, Vienna). The chopped sorghum was treated as follows:

- (1) Control, with no additives.
- (2) Inoculum A: H/M F inoculant No.9927 (Medipharm, Des Moines, IA). The inoculum contained a minimum count of 5×10^9 colony-forming units (CFU) per gram of powder of *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici*. The inoculum was suspended in 20 ml of tap water and sprayed over 15 kg of sorghum, to obtain an application rate of 10^6 CFU g⁻¹ fresh material, according to the manufacturer's instructions.
- (3) Inoculum B: Sil-All (Alltech, Wrexham, UK). The inoculum contained the same LAB as inoculum A, and an enzyme component consisting of cellulase, hemicellulase and amylase. Inoculum B was applied at the same rate as inoculum A, according to the manufacturer's instructions.

After treatment, the sorghum was ensiled in 1.5 litre Weck glass jars equipped with special lids that allow gas release only. There were 15 jars per treatment and they were stored at ambient temperature (27 ± 1 °C). Three jars per treatment were sampled for analysis on Days 1, 2, 5, 10 and 31 after ensiling. On Day 31 the silages of the various treatments were subjected to an aerobic stability test lasting 5 days in systems described by Ashbell *et al.* (1991). In this test, CO₂ production during aerobic exposure is measured as an indicator of silage deterioration.

2.2. Chemical analysis

Dry matter was determined by drying at 60 °C for 72 h; ash was obtained after 3 h at 550 °C. Water soluble carbohydrates (WSC) were determined by the phenol-sulphuric acid method,

according to Dubois *et al.* (1956). Lactic acid was determined by the colorimetric method of Barker and Summerson (1941). Volatile fermentation end-products were determined with a gas chromatograph, using a Chromosorb 101 column, over a temperature range of 140-210 °C. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963), and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970). Total nitrogen was determined by the Kjeldahl method, and volatile nitrogen by the method of Pearson and Muslemuddin (1968). Statistical analysis included a one-way analysis of variance and Duncan's multiple range test, using the SAS package (Statistical Analysis Systems, SAS, 1982).

2.3. Microbiological analysis

The microbiological analysis included the enumeration of the following populations: lactobacilli, on Rogosa agar (Oxoid CM627); yeasts and moulds, on spread plate malt extract agar (Difco), acidified to pH 3.5 with lactic acid; enterobacteria, on Violet Red Bile Glucose agar (Oxoid, CM 684), using the double layer technique; clostridial spores were estimated by the most probable number (MPN) technique on lactate-acetate agar in a miniaturized system as described by Pahlow (1986).

3. Results

Agronomical and quality characteristics of the sorghum harvested at the late bloom and soft dough stages of maturity are given in Table 1. Figures 1 to 6 show the change of pH, lactic acid and WSC during the ensiling fermentation of the sorghum at the two stages of maturity. The utilization of WSC and production of lactic acid corresponded well to changes in pH in the various silages. Both inoculants caused a faster drop in pH and faster production of lactic acid as compared with the control, and the pH of all silages was below 4.5 after only 2 days of ensiling. The WSC content in the sorghum harvested at the soft dough stage was lower than that in the sorghum harvested at the late bloom stage of maturity.

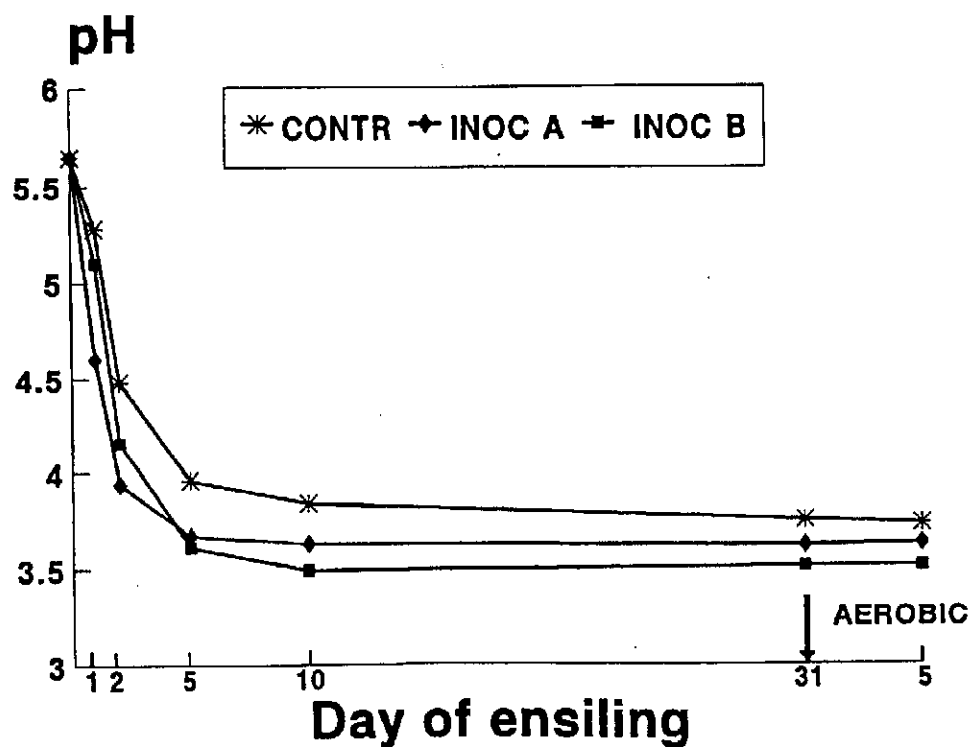
Table 1

Agronomical and quality characteristics of the forage sorghum harvested at two stages of maturity

Maturation stage	DM yield (ton ha ⁻¹)	DM (g kg ⁻¹)	Height (cm)	Harvest ¹ index (%)	IVOMD (%)
Late bloom	11.8	207	173±11	-	61.4±1.6
Soft dough	16.4	289	173±11	25	67.6±0.2

¹Harvest index is given as percentage of heads.

This may reflect the conversion of sugars into grain starch in the more mature sorghum. It was not possible to relate higher content of WSC in the silages treated with inoculum B to the enzyme component. It is possible that the higher lactic acid levels in these silages at early stages of ensiling reflect the release of WSC which were utilized in the fermentation.

**Figure 1.** pH change during ensiling of sorghum at the late bloom stage.

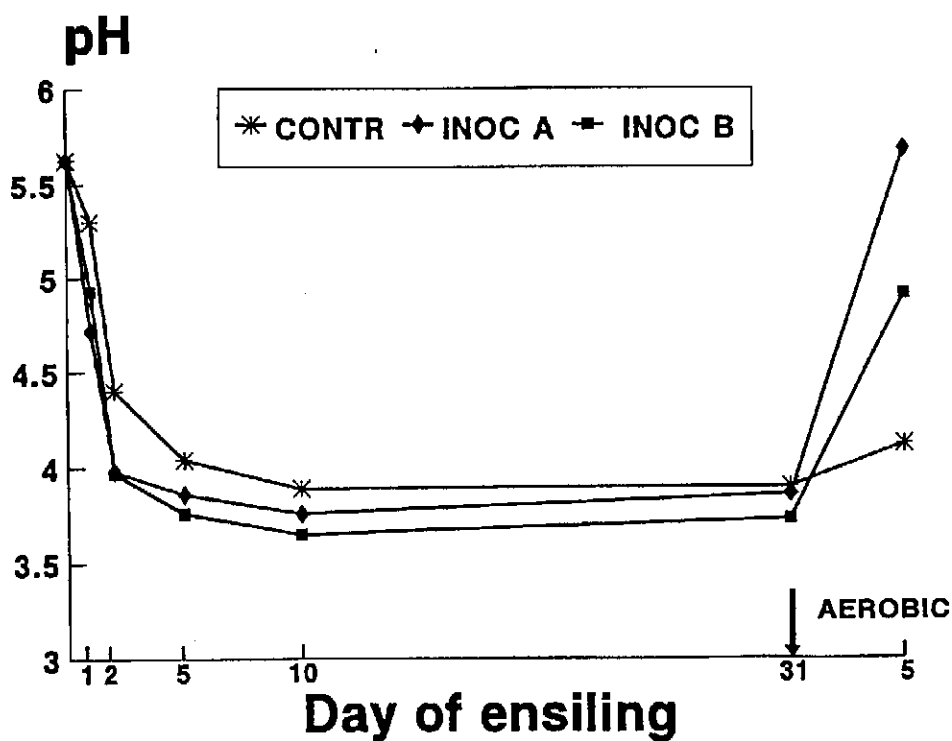


Figure 2. pH change during ensiling of sorghum at the soft dough stage.

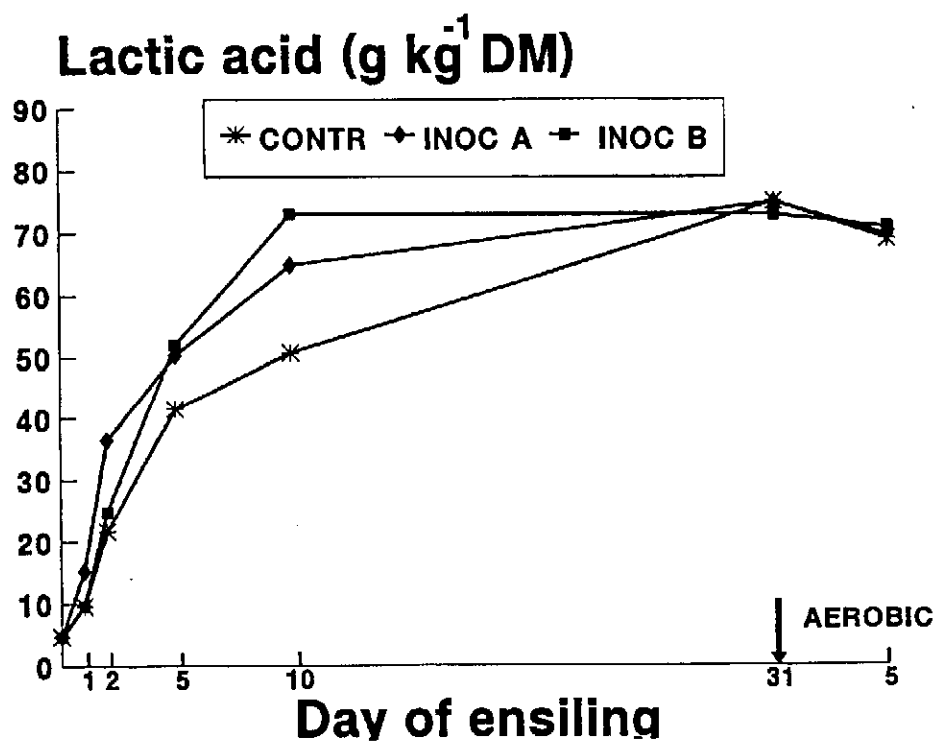


Figure 3. Lactic acid build-up during ensiling of sorghum at the late bloom stage. SEM = 5.

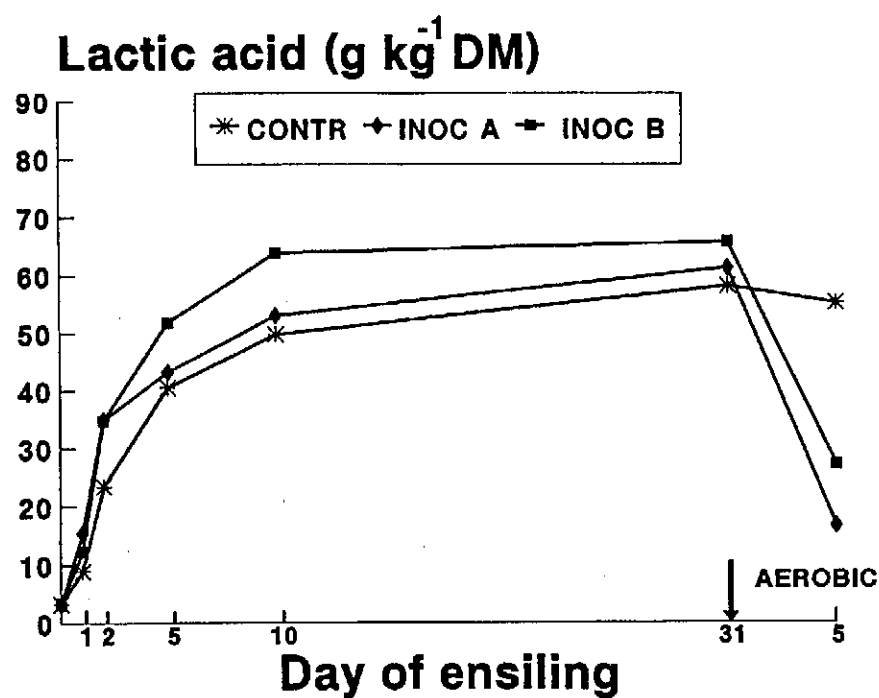


Figure 4. Lactic acid build-up during ensiling of sorghum at the soft dough stage. SEM = 3.1.

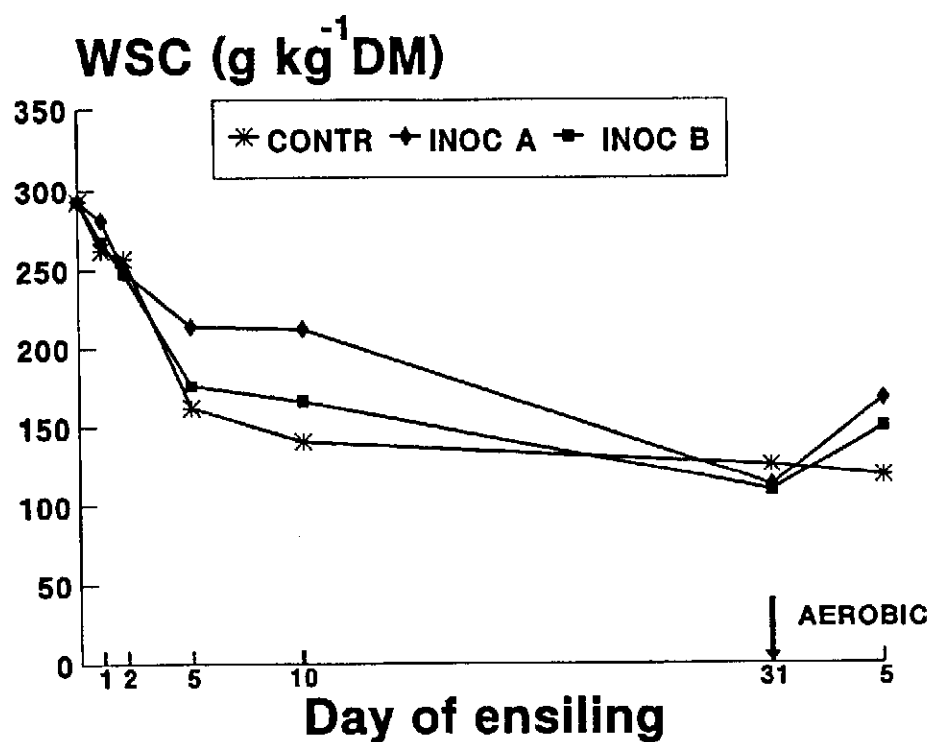


Figure 5. Change in water soluble carbohydrates during ensiling of sorghum at the late bloom stage. SEM= 19.5.

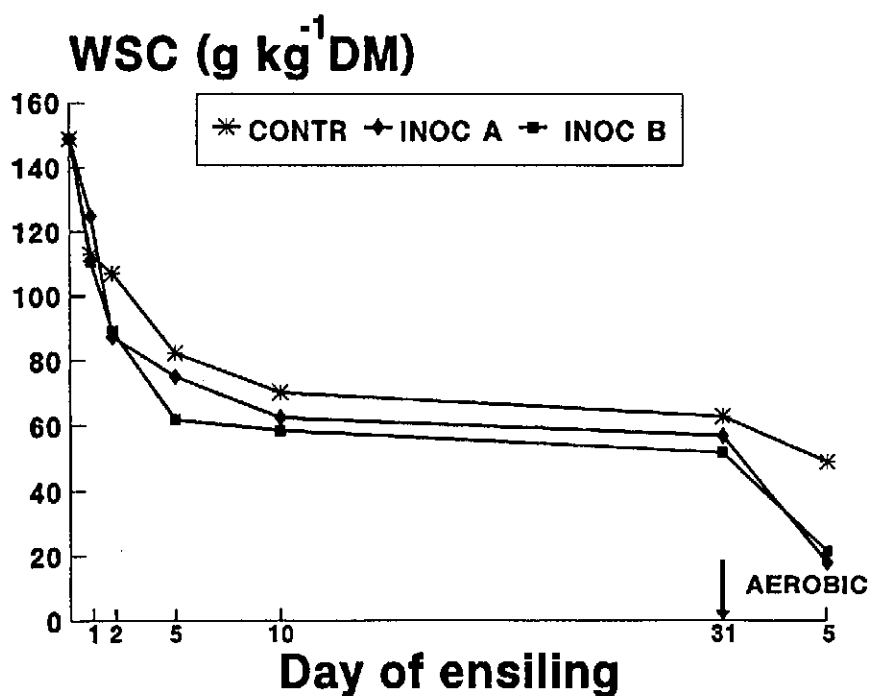


Figure 6. Change in water soluble carbohydrates during ensiling sorghum at the soft dough stage. SEM=7.7.

Table 2 gives the chemical analysis of the silages from the two stages of maturity. The fibre content in the silages of the more mature sorghum was lower than in those of the younger sorghum, which agrees with the digestibility values (Table 1).

The mean IVOMD of the silages of the soft dough stage was $65.6 \pm 0.7\%$, with no significant differences between treatments.

After 5 days of aerobic exposure, the IVOMD was 64.3%, 60.7% and 60.8% for the control and inoculant A and B treated silage respectively. The decrease in the IVOMD of the inoculated silages of the soft dough stage upon aerobic exposure reflects their intensive deterioration (Table 3).

Table 2

Chemical analysis of forage sorghum silages (Day 31) ensiled at the late bloom and soft dough stage of maturity

Maturation stage	Analysis (g kg ⁻¹ DM)	Control	Treatment		SEM
			Inoculant A	Inoculant B	
Late bloom	DM	214	212	213	4.4
	Ash	85	85	85	8
	NDF	578	595	584	11
	ADF	348	358	347	6
	ADL	44.1 ^{ab}	44.6 ^b	39.3 ^a	1.5
	Total N	17.0	16.3	16.3	0.4
	Ammonia-N ¹	1.7 ^a	1.1 ^b	0.9 ^b	0.1
	Ethanol	10	7	2	2.7
	Acetic acid	13	ND	ND	2
Soft dough	DM	276	276	284	4.7
	Ash	82	81	80	1.7
	NDF	548 ^{ab}	520 ^a	576 ^b	16
	ADF	288	285	280	5
	ADL	38	35	36	2
	Total N	13.1	13.4	14.0	0.3
	Ammonia-N ¹	0.9	1.0	0.8	0.1
	Ethanol	6	8	7	2
	Butyric acid	2	ND	ND	3

¹Ammonia-N is given in grams per 100 of total N. ND= not detected. Within rows, means with the same superscript do not differ significantly ($P < 0.05$).

Table 3Results of the aerobic stability test (CO₂ production; g kg⁻¹ DM)

Treatment	Late bloom	Soft dough
Control	1.4 ^a	15.5 ^a
Inoculant A	1.6 ^a	48.8 ^c
Inoculant B	2.1 ^a	37.1 ^b

Within columns, means with the same superscript do not differ significantly ($P < 0.05$).

The inoculants did not have a major effect on any of the chemical parameters that were measured. The major fermentation end-product was ethanol, probably indicating yeast activity. In the control silages of the young sorghum, acetic acid was also detected.

Table 3 gives the results of the aerobic stability test. Silages of all treatments of the young (late bloom) sorghum were stable upon exposure to air. The more mature (soft dough) sorghum was less stable, as indicated by larger amounts of CO₂ produced during aerobic exposure. However, the inoculated silages of this stage of maturity produced significantly more CO₂ as compared with the control, indicating more intensive spoilage processes. During spoilage there was an increase in pH, a decrease in lactic acid content (Figs. 2 and 4, respectively), and a decrease in digestibility.

Tables 4 and 5 give the microbiological analysis of the sorghum silages of the two stages of maturity. In all silages the number of lactobacilli increased after only 1 day of ensiling. Enterobacteria and clostridia were eliminated in early stages of ensiling, as a result of the low pH values. The numbers of yeasts and moulds in the sorghum silages of the late bloom stage were low, even upon exposure to air. However, yeast numbers were higher in the sorghum silages of the soft dough stage and increased upon aerobic exposure. Moulds were found in the aerobically exposed silages of inoculum B. The microbiological profile agrees with CO₂ values (Table 3), and it seems that yeasts are the major spoilage organisms during aerobic exposure of mature sorghum silages.

Table 4

Microbiological examination (log CFU g⁻¹ DM) of forage sorghum ensiled at the late bloom stage with and without addition of bacterial inoculants

Treatment	Time	LAB	E	Y	M	CL
Fresh material	Day 0	4.4	7.2	5.9	NF	2.1
<i>Control</i>						
Silage	Day 1	8.9	8.6	3.1	3.5	4.8
	Day 2	9.8	7.9	3.4	1.7	NF
	Day 5	9.7	5.0	NF	NF	NF
	Day 10	9.1	NF	NF	NF	NF
	Day 31	7.2	NF	NF	2.4	NF
Aerobic	Day 5	6.0	NF	2.4	3.1	NF
<i>Inoculant A</i>						
Silage	Day 1	9.6	8.2	3.5	2.9	3.2
	Day 2	9.9	5.8	3.7	NF	NF
	Day 5	9.9	NF	NF	2.4	NF
	Day 10	8.9	NF	NF	NF	NF
	Day 31	6.3	NF	NF	NF	NF
Aerobic	Day 5	5.2	NF	6.5	3.5	1.6
<i>Inoculant B</i>						
Silage	Day 1	9.5	8.7	3.7	3.9	3.5
	Day 2	9.9	6.1	2.4	NF	NF
	Day 5	9.9	NF	NF	NF	NF
	Day 10	9.5	NF	NF	NF	NF
	Day 31	7.1	NF	NF	NF	NF
Aerobic	Day 5	7.3	NF	9.3	NF	NF

E, enterobacteria; Y, yeasts; M, moulds; CL, clostridial spores; NF, not found.

Table 5

Microbiological examination (log CFU g⁻¹ DM) of fodder sorghum ensiled at the soft dough stage with and without the addition of bacterial inoculants

Treatment	Time	LAB	E	Y	M	CL
Fresh material	Day 0	4.8	7.3	6.7	7.3	2.5
<i>Control</i>						
Silage	Day 1	9.2	9.5	5.9	6.7	3.7
	Day 2	9.1	8.5	5.8	5.5	NF
	Day 5	10.2	5.9	6.2	4.5	NF
	Day 10	9.2	6.6	5.3	NF	NF
	Day 31	7.9	NF	7.0	NF	NF
Aerobic	Day 5	9.1	NF	9.2	NF	NF
<i>Inoculant A</i>						
Silage	Day 1	9.8	8.7	6.0	6.1	NF
	Day 2	10.0	7.5	7.1	5.3	NF
	Day 5	9.8	4.5	4.3	2.2	NF
	Day 10	10.5	5.8	6.5	NF	NF
	Day 31	7.3	NF	6.7	NF	2.5
Aerobic	Day 5	10.1	NF	10.1	8.2	NF
<i>Inoculant B</i>						
Silage	Day 1	9.5	8.8	6.4	6.7	2.4
	Day 2	9.8	6.5	4.7	3.1	2.1
	Day 5	9.8	4.7	3.6	2.8	NF
	Day 10	9.6	4.1	4.9	NF	NF
	Day 31	6.7	NF	6.0	NF	NF
Aerobic	Day 5	9.7	NF	9.9	NF	NF

E, enterobacteria; Y, yeasts; M, moulds; CL, clostridial spores; NF, not found.

4. Discussion

In general, as maturity of sorghum plants advances, digestibility decreases. This trend was observed in various studies (Fox *et al.*, 1970; Black *et al.*, 1980; Dannhauser *et al.*, 1990). An inverse relationship between cell wall constituents and digestibility was exhibited in various

sorghum cultivars, and when cell wall content is above 55% digestibility decreases (Danley and Vetter, 1973). This is true in no-heading sorghum cultivars, the cell wall contents of which increases with maturity. However, in high grain producing sorghum there is a dilution effect of the fibrous components, owing to starch formation in the grain as maturity advances (Owen and Webster, 1963; Hart, 1990). In this regard, differences between and within cultivars also exist (Dickerson, 1986). In the current experiment, harvesting the FS2 sorghum cultivar at the soft dough stage (harvest index 25%) resulted in a higher DM yield than at the late bloom stage. The digestibility of the sorghum increased from the late bloom to the soft dough stage. The NDF, ADF and ADL contents of the sorghum silage were lower at the dough than at the late bloom stage. These findings agree with the results of Owen and Webster (1963) and Hart (1990).

The ensiling process of the sorghum from either stage of maturity was rapid under laboratory conditions. This was due to adequate WSC content and low buffer capacity of the sorghum, which allowed rapid development of lactobacilli, rapid and intensive production of lactic acid, and a fast decrease in pH. However, the more mature sorghum was less stable upon aerobic exposure than were the sorghum silages of the younger plants. The silages of the more mature sorghum for all treatments contained more yeasts than those of the young crop, which may explain the instability of the former. Yeasts can play a major role in the aerobic deterioration of silages (Seale, 1986).

The inoculated silages of the sorghum of the soft dough stage were the least stable upon aerobic exposure. The impairment of the aerobic stability of certain silages by LAB inoculants has been observed also in other studies (Rust *et al.*, 1989; Weinberg *et al.*, 1993). It could be that intensive production of lactic acid by the LAB inoculants enhances the activity of lactate-assimilating yeasts during aerobic exposure. In this study inoculants affected the yeast numbers after aerobic exposure only in the silages of the late bloom stage (Table 4), and not those of the soft dough stage, where the numbers were very high in all treatments. The reason for enhanced aerobic deterioration of the inoculated silages of the soft dough stage is not yet clear and should be studied further.

Because of the adequate ensiling process, and the adverse effect of inoculants on the aerobic stability of the silage, the need for inoculants and enzymes for such crops is questionable.

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Chapter 5

The ensiling of maize.

A comparison of the yield, nutritional value and predicted production potential of different maize hybrids for silage production

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The effect of a lactic acid bacterial inoculant on maize silage

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The effect of the addition of a lactic acid bacterial inoculant with an enzyme to maize at ensiling on silage composition, silage intake, and milk production

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Introduction

Maize is the major silage crop in the world because of its high yield, high energy concentration and low cost. Maize is regarded as an ideal silage crop with a relative high dry matter content, low buffering capacity and adequate levels of water soluble carbohydrates (McDonald, *et al.*, 1991). However, quality problems in maize silage may occur and this has been related to high somatic cell counts in milk (Ann, *et al.*, 1999). Ann *et al.* (1999) has reported that *Penicillium roqueforti* has been found in late cut maize silage and that a field-study on hygienic quality in maize silage is running in Scandia, Sweden. The poor aerobic stability of maize silage as well as the improvement of grain utilization in maize silage, still require further research (Wilkins *et al.*, 1999). Newly selected bacterial strains are available that will improve aerobic stability of maize silage (Harrison *et al.*, 1999).

For harvesting whole-plant maize, machines with an on-board kernel processor have drawn more and more attention, especially from dairy farmers in North America (Shinner, 1997). The kernel processor helps to minimize the risks associated with hybrids that increase rapidly in dry matter content with maturity. Higher milk production by dairy cattle (Bal *et al.*, 2000; Harrison *et al.*, 1997) and improved gains in growing cattle (Young *et al.*, 1998) have been reported when kernel processed maize silage was fed. In South Africa very few silage choppers with kernel processors are in operation and many farmers often do not have access to contractors. Most farmers use single or double row silage choppers and may take from one to three weeks to fill and seal a silage bunker depending on the size of the bunker and equipment available. Conditions for growth of yeasts and moulds are therefore often favourable. This increases the risk of aerobic instability and heating of silage during the feeding out phase as yeasts play a prominent role in aerobic deterioration of silages (Woolford and Wilkie, 1984).

In South Africa only three to four silage inoculants are marketed. Maize silage is the major silage crop in South Africa and farmers are often recommended to apply inoculants to maize at ensiling. Honig and Daenicke (1993) have shown that even in easily ensilable maize, fermentation losses can be further reduced by the use of an inoculant. The average daily gain of bulls was increased

but the aerobic stability of the maize silage was reduced by one day in inoculated maize silage compared to untreated control silage. The adding of homofermentative lactic acid bacteria such as *Lactobacillus plantarum* can produce a silage that is unstable when exposed to air (Kung, *et al.*, 1991; Rust *et al.*, 1989; Weinberg, *et al.*, 1993). Therefore aerobic stability of maize silage should always be determined when additives are evaluated.

The next three papers deal with the nutritional value of maize silages made from different maize hybrids and evaluates the effect of two lactic acid bacterial inoculants on maize silage.

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A comparison of the yield, nutritional value and predicted production potential of different maize hybrids for silage production

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Abstract

The yield, nutritional value and production potential of silage made from twenty one maize hybrids was compared. The digestibility of organic matter and predicted intake, mean retention time and milk production potential were found to differ between hybrids ($p < 0.05$). Acid detergent fibre content could not be used to accurately predict the metabolisable energy content of silage.

Keywords: Milk production, intake, mean retention time, silage, yield.

1. Introduction

Maize silage is used extensively in diets for beef and dairy cattle in South Africa. Large differences in nutritional value may exist between silage made from different maize hybrids (Hunt *et al.*, 1993). Givens *et al.* (1995) have shown that substantial variation exists in respect of the proportions of starch and cell-wall components in maize silage, and that these are not closely related to differences in digestibility. Methods used to characterize the nutritional value of maize silage should take differences in the rates and extent of rumen degradation of starch and cell-wall components into account (Givens *et al.*, 1995). Aufrère *et al.* (1992) evaluated 12 maize hybrids and found that crude protein content varied from 5.8 to 13% and organic matter digestibility from 62.8 to 77.4%. This variation is influenced by the hybrid used, stage of growth, location and

season. The aim of the present study was to compare yield, nutritional value, rate and extent of digestion, predicted intake and milk production potential of silage made from 21 different maize hybrids.

2. Materials and methods

Twenty one maize hybrids were planted using a randomized block design. Three adjacent blocks, each containing a 5 m row of each hybrid, were planted at a density of 22 000 plants/ha at Oberholzer, South Africa (26° 08' S, 27° 35' E; altitude 1725 m). Rows were 91cm apart. The soil was a sandy loam Hutton type with a pH(KCl) of 4.8; the concentrations of P, K, Ca, Mg, Zn and Na were 45, 75, 520, 87, 5.2 and 12 mg/kg respectively. The total rainfall from October to May was 482 mm, the monthly precipitation (mm) being 58, 35, 145, 79, 28, 81, 40 and 16 for October, November, December, January, February, March, April and May respectively. The long-term average rainfall for the area is 561 mm per year. Fertilizer (2:3:4(33)) was applied at the time of planting at a rate of 75 kg/ha. One hundred kg KAN (28) was applied six weeks after planting, when plants had reached the 6-8 leaf stage. All maize hybrids were harvested at the three-quarter milkline stage: when the kernels were dented and the lower leaves of the plants had started to dry off.

Plants were counted and weighed to determine yield. All plants were passed through a commercial silage chopper, and a representative sample of the fresh chopped material was taken. The plant-material was ensiled in mini-silos, which consisted of 1.5 litre glass jars equipped with lids that allowed gas release (J. WECK, GmbH, Wehr-Oflingen, Germany). Dry matter (DM) content was determined by drying at 60° C for 72 hours. *In vitro* organic matter digestibility (IVOMD) of oven-dried samples and rate of digestion were determined as described by Tilley and Terry (1963) and modified by Pienaar and Kühn (1991) to include measurement of gas production. Metabolisable energy (ME) content was estimated using IVOMD and fat content as follows:

$$\text{Total digestible nutrients (TDN)} = 48 \text{ h IVOMD} + ((\% \text{ fat}-2)/100) \times 1.91$$

$$\text{ME (MJ/kg DM)} = \text{TDN} \times 20.66 \times 0.82 \times \% \text{OM} / \% \text{DM}$$

Intake was calculated by dividing the predicted rumen organic matter (OM) content by the predicted retention time of OM in the rumen as described by Pienaar and Roux (1989). The predicted milk production potential of hybrids for a 600 kg cow was calculated from the estimated ME value and intake, assuming that no other nutrient was limiting (Pienaar and Roux, 1989). Neutral detergent fibre (NDF) was determined according to Robertson and Van Soest (1981), and acid detergent fibre (ADF) according to Goering and Van Soest (1970). Nitrogen (Kjeldahl) and fat content were determined according to AOAC (1984). Analysis of variance was done using the Statgraphics (1988) statistical package to determine least significant differences.

3. Results and discussion

The composition of silage made from the different maize hybrids is given in Table 1. All silage samples had a low pH and were well preserved. There were no differences in NDF or ADF content between hybrids. Mean NDF and ADF values were $46.7 \pm 1.9\%$ and $25.2 \pm 1.1\%$ respectively, and are comparable with the values of $47.7 \pm 3.6\%$ for NDF and $23.3 \pm 2.6\%$ for ADF reported by Aufrère *et al.* (1992) for French cultivars.

The estimated ME values of silage differed between cultivars ($p < 0.05$), and varied from 8.83 to 10.20 MJ ME/kg DM. De Boever *et al.* (1988) reported that the ME content of 50 maize silages in Belgium varied between 9.75 and 12.21 MJ ME/kg DM. The crude protein content (Table 1) of maize silage varied between 5.7 and 7.7%, which was lower than values (8.2-11.4%) reported by De Boever *et al.* (1988). The relationship ($p < 0.05$; $R^2 = 0.48$; $n = 66$) between ME and ADF content was best described by the equation:

$$\text{ME (MJ/kg)} = 14.0501 - 0.18421 \text{ ADF (\%)}$$

The standard error of the constant was 0.6001, and the standard error of the factor was 0.023737.

Givens *et al.* (1995) compared the use of different laboratory analyses to predict the *in vivo* digestibility of maize silage, and found that ADF concentration accounted for 42.6% of the variation in organic matter *in vivo* digestibility.

Table 1

Nutritional value and pH of silage made of different maize hybrids (DM basis)

Hybrid	DM (%)	pH	CP (%)	IVOMD (%)	ME (MJ/kgDM)	NDF (%)	ADF (%)
NS9100	35.0 ^{abcd}	3.53 ^a	6.6 ^{ab}	72.4 ^a	10.20 ^a	46.3	23.6
PAN6364	39.7 ^a	3.60 ^{abcd}	6.7 ^{ab}	65.9 ^{bc}	10.00 ^{ab}	45.7	23.9
PAN6140	33.0 ^{abcd}	3.66 ^{abcdef}	7.4 ^a	63.3 ^{ab}	9.80 ^{abcd}	46.3	25.3
CAR3414	31.5 ^{cd}	3.66 ^{abcdef}	6.3 ^{ab}	64.0 ^{ab}	9.87 ^{abc}	49.4	25.5
PHB3253	33.6 ^{bcd}	3.65 ^{abcde}	6.7 ^{ab}	62.0 ^{abc}	9.73 ^{abcd}	44.3	22.9
SNK2266	34.1 ^{bcd}	3.81 ^{efghi}	6.7 ^{ab}	61.7 ^{abcd}	9.50 ^{abcde}	48.1	25.4
CAR3852	35.3 ^{abcd}	3.62 ^{abcd}	7.2 ^{ab}	62.7 ^{abc}	9.70 ^{abcde}	44.1	24.3
SNK2888	36.0 ^{abc}	3.74 ^{cdefghi}	7.2 ^a	63.7 ^{ab}	9.70 ^{abcdef}	45.0	23.8
A1598	37.5 ^{ab}	3.75 ^{cdefghi}	7.2 ^{ab}	62.3 ^{abc}	9.63 ^{abcdef}	46.3	24.9
SNK2154	35.5 ^{abc}	3.91 ⁱ	6.7 ^{ab}	60.7 ^{abcd}	9.37 ^{abcdef}	46.4	25.8
NS5122	30.5 ^d	3.65 ^{abcde}	7.1 ^{ab}	61.7 ^{abcd}	9.30 ^{abcdef}	48.2	25.4
PHB3442	33.0 ^{bcd}	3.87 ^{hi}	7.2 ^a	60.3 ^{abcd}	9.33 ^{abcdef}	49.5	26.6
PAN6146	34.8 ^{bcd}	3.76 ^{defghi}	6.6 ^{ab}	60.7 ^{abcd}	9.40 ^{abcdef}	50.1	26.1
PAN6479	31.4 ^{cd}	3.58 ^{abc}	5.7 ^b	61.0 ^{abcd}	9.50 ^{abcdef}	45.7	23.4
PHB3412	31.5 ^{cd}	3.70 ^{bcddefgh}	7.2 ^a	59.3 ^{bcd}	9.13 ^{bcddef}	45.3	24.9
SNK2255	34.9 ^{bcd}	3.83 ^{ghi}	7.1 ^{ab}	59.0 ^{bcd}	9.13 ^{bcddef}	46.2	25.6
CAR4526	33.7 ^{bcd}	3.71 ^{cdefgh}	7.1 ^{ab}	62.0 ^{abcd}	9.23 ^{bcddef}	45.8	26.5
SNK2665	31.3 ^{cd}	3.53 ^{ab}	7.7 ^a	61.0 ^{abcd}	9.17 ^{bcddef}	48.1	26.6
A1539	32.5 ^{cd}	3.83 ^{fghi}	7.3 ^a	58.0 ^{cd}	8.93 ^{cdef}	48.0	26.1
SNK2265	33.1 ^{bcd}	3.67 ^{abcdefg}	6.4 ^{ab}	58.0 ^{cd}	8.90 ^{def}	48.4	26.3
PAN6255	33.7 ^{bcd}	3.86 ^{hi}	6.9 ^{ab}	56.7 ^d	8.73 ^f	43.0	25.4
PAN6043	34.0 ^{bcd}	3.83 ^{fghi}	7.0 ^{ab}	57.7 ^{cd}	8.83 ^{ef}	47.8	26.0
Means	33.9	3.72	6.9	61.1	9.41	46.7	25.2
SD	±2.2	±0.11	±0.4	±2.3	±.43	±1.9	±1.1
SEM	1.7	0.06	0.3	1.8	0.33	2.7	1.4

^{abcdef} Means within columns without common superscripts differ ($P < 0.05$); SD= Standard deviation
SEM = Standard error of means.

Table 2

Yield and predicted intake (600kg cow), mean retention time and milk production potential of silage made from different maize hybrids.

Hybrid	DM Yield (ton/ha)	DM intake (kg/day)	Mean retention time (h)	Milk production (kg/day)
NS9100	14.7 ^a	13.4 ^{bcd}	24.9 ^{abc}	14.9 ^{cde}
PAN6364	13.2 ^{ab}	12.4 ^{ab}	27.0 ^{bcd}	14.2 ^{abcd}
PAN6140	12.0 ^{ab}	13.1 ^{bcd}	25.7 ^{abc}	14.0 ^{bcd}
CAR3414	14.7 ^a	12.8 ^{abcd}	26.1 ^{abc}	13.5 ^{abcd}
PHB3253	11.3 ^{ab}	12.8 ^{abcd}	26.1 ^{abc}	13.1 ^{abcd}
SNK2266	9.7 ^b	13.5 ^{cd}	24.6 ^{ab}	15.3 ^{bcd}
CAR3852	10.1 ^b	12.8 ^{abcd}	26.0 ^{abc}	13.3 ^{abcd}
SNK2888	12.1 ^{ab}	12.4 ^{abc}	26.8 ^{bcd}	12.3 ^{abcd}
A1598	11.5 ^{ab}	12.9 ^{abcd}	25.8 ^{abc}	14.1 ^{bcd}
SNK2154	11.1 ^{ab}	13.8 ^d	24.3 ^a	15.9 ^{bcd}
NS5122	10.7 ^{ab}	12.9 ^{abcd}	26.1 ^{abc}	13.4 ^{abcd}
PHB3442	11.1 ^{ab}	13.0 ^{bcd}	25.7 ^{abc}	13.3 ^{abcd}
PAN6146	13.0 ^{ab}	12.6 ^{abc}	26.7 ^{abcd}	11.7 ^{abc}
PAN6479	12.9 ^{ab}	13.0 ^{bcd}	25.4 ^{abc}	15.0 ^{bcd}
PHB3412	12.2 ^{ab}	12.8 ^{abcd}	26.1 ^{abc}	13.2 ^{abcd}
SNK2255	10.7 ^{ab}	13.1 ^{bcd}	25.3 ^{abc}	14.6 ^{bcd}
CAR4526	10.8 ^{ab}	11.8 ^a	28.6 ^{cde}	9.5 ^a
SNK2665	10.4 ^{ab}	12.5 ^{abc}	27.0 ^{abcd}	11.6 ^{ab}
A1539	10.9 ^{ab}	13.5 ^{cd}	24.6 ^{abc}	16.2 ^{bcd}
SNK2265	10.4 ^b	12.3 ^{ab}	27.0 ^{cd}	11.6 ^{abc}
PAN6255	10.6 ^{ab}	13.1 ^{bcd}	25.4 ^{abc}	13.2 ^{abcd}
PAN6043	11.6 ^{ab}	13.0 ^{bcd}	25.6 ^{abc}	13.6 ^{abcd}
Means	11.7	12.9	25.9	13.6
SD	±1.3	± 0.45	±1.0	±1.6
SEM	1.4	0.4	0.8	1.5

^{abcdef} Means within columns without common superscripts differ ($p < 0.05$); SD= standard deviation; SEM= standard error of the means.

Prediction of the energy value of maize silage from ADF concentration may result in under or over estimation because the digestibility of cellulose or hemicellulose may vary, depending on the degree of crystallinity, acetylation or interlinking with lignin and silica (Giger-Reverdin, 1995).

Silage yield, predicted intake, mean retention time and milk production potential is shown in Table 2. The predicted intake of SNK 2154, i.e. 13.8 kg DM, was greater ($p < 0.05$) than that of PAN 6364, SNK 2888, PAN 6146, CAR 4526, SNK2665 or SNK 2265.

The DM yield of NS 9100 and CAR 3414 was higher than that of SNK 2266, CAR 3852 or SNK2265 ($P < 0.05$). Variation in the number of plants per row was the main cause of yield differences between hybrids. There were eight plants per five metre row for SNK 2266, CAR 3852 and SNK 2265, which was less ($p < 0.05$) than that for NS 9100 or PAN 6043 (10 plants/5m). An average of 9.67 plants/5m was recorded for CAR3414. Differences between predicted dry matter intakes were due to differences between predicted mean retention times. Maize hybrid SNK 2154 had a shorter predicted mean retention time (24.3 h) than that of PAN 6364, SNK 2888, CAR 4526 or SNK 2255 (Table 2). The estimate of mean retention time (MRT) combines the effects of digestibility and rate of digestion (Pienaar and Roux 1989). A shorter predicted MRT implies a higher digestibility and/or a faster rate of digestion. Predicted milk production varied from 9.5 to 16.2 kg/d. Differences in predicted milk production potential between hybrids were caused by differences in digestibility, predicted mean retention time and predicted intake. The production potential of maize hybrids should be considered when choosing a maize hybrid for silage production.

It was concluded that maize hybrids differ in ME content, rate of digestion, predicted intake and predicted milk production potential. The content of NDF and ADF did not differ between the maize cultivars used in this study and could therefore not be used to predict nutritional value or production potential.

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The effect of a lactic acid bacterial inoculant on maize silage

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Abstract

Maize silage is an important forage in South Africa and it is often made without the use of any additives. Very little information on the fermentation dynamics of maize silage under local conditions is presently available. The aim of this study was to determine if the adding of a lactic acid bacterial inoculant to maize at ensiling will improve the fermentation dynamics of maize silage and to determine the effect of the inoculant on the intake and growth of lambs. Maize was harvested at the hard dough stage with a Feraboli 930 silage chopper and ensiled with or without the addition of a lactic acid bacterial inoculant in 18 laboratory silos for each treatment. Silos were 1.5 litre glass jars, equipped with a special lid with springs which enables gas release only. The fermentation dynamics during ensiling was determined by opening three silos on day 1, 2, 4, 10 and 95 of ensiling. The adding of the inoculant to maize at ensiling did not result in a more rapid drop in pH and higher levels of lactic acid. For the intake and growth study maize was ensiled in thirty 210 litre drums lined with a plastic bags for each treatment. The material was compacted, the bags were closed and a concrete paving stone (20 kg) was placed on top of the bag. The intake and growth of South African Mutton Merino lambs fed inoculated and untreated maize silage was determined. Two groups of 14 lambs weighing 26.4 ± 1.9 and 26.4 ± 1.6 kg were randomly allocated to the control and the inoculant treatment, respectively. The average daily gain of lambs fed a diet consisting of either 60% control or inoculated maize silage over a growth period of 60 days was 239 ± 26 and 255 ± 44 g day⁻¹, respectively. The feed conversion efficiencies were 4.94 and 4.93 kg DM feed kg⁻¹ liveweight gain for lambs fed the control and inoculated silage diets, respectively. Although the laboratory study showed very little effect of

adding a lactic acid bacterial inoculant to maize at ensiling, lambs tended to consume more of the inoculated silage.

Keywords: Maize silage: Fermentation: Inoculant: Intake: Growth: Lambs

1. Introduction

Maize (*Zea mays*) is the most popular cereal crop conserved as silage in many parts of the world (McDonald *et al.*, 1991). It is regarded as an ideal silage crop with a relative high dry matter (DM) content, low buffering capacity and adequate water soluble carbohydrates for fermentation to lactic acid. Very little information is available on the epiphytic microflora of forage crops in South Africa. Insufficient numbers of viable homofermentative lactic acid bacteria on a crop at harvesting could result in a delay in the drop of pH of plant material during ensiling, higher loss of nutrients and silage with a poor intake (Woolford, 1984). The adding of homofermentative lactic acid bacteria to a crop at ensiling will ensure that sufficient viable lactic acid bacteria are present (Woolford, 1984). Although problems with the ensiling of maize are seldom encountered in South Africa the adding of inoculants might improve the silage quality. The aerobic stability of silage may be adversely affected by the addition of bacterial inoculants (Rust *et al.*, 1989). It is therefore important to determine if the inoculant has an effect on the aerobic stability of the silage.

The aim of the study was to determine the effect of a lactic acid bacterial inoculant on the fermentation dynamics during ensiling and the aerobic stability of maize silage. The intake and growth of lambs consuming diets containing 60% untreated or inoculated maize silage was determined.

2. Materials and methods

2.1. Laboratory study

Maize, variety Senkuil was planted on the 24th of November 1993 at the Animal Nutrition and

Animal Products Institute, Irene (longitude 28° 13'S; latitude 25° 55'E, altitude 1524 m). The plant density was 38095 plants per hectare. Maize was harvested at the hard dough stage with a Feraboli 930 silage chopper. The chop length was determined by measuring twelve randomly picked pieces of stem. A representative sample of fresh chopped maize was taken and frozen at -20°C. The maize was ensiled with or without the addition of a lactic acid bacterial (LAB) inoculant in 1.5 litre Weck glass jars (Weck, Wehr-Oflingen, Germany). The glass lid is fastened with metal clamps which enables gas release when pressure builds up in the jar. The LAB inoculant contained *Lactobacillus plantarum*, *L. bulgaricus* and *L. acidophilus* together with the enzymes amylase and cellulase. The inoculant was applied by dissolving 0.2 g of freeze dried inoculant into 80 ml of water and spraying this onto 20 kg of chopped maize to provide 10⁶ colony forming units (CFU) of lactic acid bacteria per gram of fresh material.

Eighteen 1.5 litre mini silos were filled for each treatment to follow the fermentation dynamics during the ensiling period. The silos were stored at a minimum temperature of 18.1 ± 4.1°C and a maximum temperature of 21.9 ± 2.9°C. Three silos were opened for each treatment on each of day 1, 2, 4, 10 and 95 after ensiling. Representative samples from these silos were taken for DM determination and chemical analysis (stored at -20°C). Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 h. Water soluble carbohydrate (WSC), pH and lactic acid (LA) were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 min with a stomacher. Water soluble carbohydrates were determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956) and lactic acid was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acid (VFA) was determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35 m X 3 mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H₃PO₄). The column was conditioned for 48 h at 165°C with a nitrogen carrier gas flow of 40 ml min⁻¹. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963) and neutral detergent fibre (NDF) according to Van Soest *et al.* (1991). Total nitrogen was determined by the Kjeldahl method. The ammonia nitrogen content of silage was determined by homogenizing 50 g of silage in 250 ml of a 0.1 N H₂SO₄ solution for 3 min. The homogenate was filtered through Whatman No.4 filter paper and the ammonia content

in the filtrate was determined by distillation using a Buchi 342 apparatus and a Metröhm 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen.

At day 95 of ensiling, the silage was exposed to air for 5 days and the aerobic stability determined according to the method of Ashbell *et al.* (1991). According to this method, CO₂ production during the aerobic deterioration is measured as an indicator of aerobic spoilage of silage.

Microbiological analysis was carried out on the fresh chopped material and the silages. Forty gram of material was weighed into sterile stomacher bags, 360 ml of sterile saline water added and the sample was homogenized by stomaching for 3 min. The extract was further diluted and used for microbiological analyses. Enumeration of lactobacilli was done using Rogosa agar (Oxoid CM627) supplemented with 0.4 g l⁻¹ of cycloheximide to inhibit yeast growth. Agar plates were incubated at 30°C. Enterobacteria were enumerated on violet-red bile glucose agar (Oxoid CM684) using the double layer technique (incubated at 37°C) and yeasts and moulds were enumerated on malt extract agar (Difco) adjusted to pH 3.5 by the addition of 50 ml of 10% lactic acid per litre (incubated at 25°C). Colonies were counted directly on the agar plates. Clostridial spores were determined by the most probable number technique on lactate-acetate agar as described by Pahlow (1986).

2.2. Growth study

The control treatment was ensiled without any additives and inoculant was applied to the maize on the silage chopper (Feraboli 930) at a rate of 2 litre per tonne of fresh chopped maize (10 g of inoculant was dissolved in 2 l water 4 h before application). The silage chopper was set to give a chop length of 7 mm. Chopped maize was ensiled in thirty 210 litre drums lined with a plastic bags for each treatment. The material was compacted, the bags were closed and a concrete paving stone (20 kg) was placed on top of the bag. The drums were closed with a steel lid to prevent damage to the bags by rodents. After 125 days of ensiling, the silages were fed ad libitum to South African Mutton Merino wethers housed in separate stalls. Fourteen lambs were randomly allocated to each treatment with an average weight of 26.4 ± 1.9 and 26.4 ± 1.6 kg for control

and the inoculant treatment, respectively.

Lambs were vaccinated against pulpy kidney and pasteurella, dewormed and a growth stimulant, Ralgro containing 73.8% zeranol was implanted at back of the ear at 12 mg per lamb. Four hundred gram of a concentrate containing (on a DM basis) 61.9% maize, 29.7% sunflower oilcake, 2.48% feed lime, 1.24% salt, 1.24% urea and 0.5% vitamin and mineral premix was fed during the first week of the growth trail. For the rest of the growth trail 500 g of the concentrate was fed daily to each lamb. Lambs consumed all the concentrate and silage residues were removed, weighed and oven dried daily at 90°C for 24 h. Lambs were weighed monthly and slaughtered at 40 ± 2 kg.

Data of the laboratory and growth studies were processed by one way analysis of variance using Statgraphics (1988).

3. Results

3.1. Laboratory study

The average chop length of the stem of maize plants was 5.0 ± 0.7 mm ($n = 12$). The change in pH, lactic acid and WSC of maize during the ensiling period is given in Figs. 1-3, respectively.

The chemical composition of silages after 95 days of ensiling is given in Table 1. Both the control and the inoculated silages were well preserved as indicated by the low pH and the small amount of ammonia present.

The counts of colony forming units of lactic acid bacteria, enterobacteria, yeasts and moulds and most probable number of clostridial spores are given in Table 2. High numbers of lactic acid bacteria were present on the untreated maize before ensiling and the control silage did have a higher number of clostridial spores than the inoculated silage (Table 2).

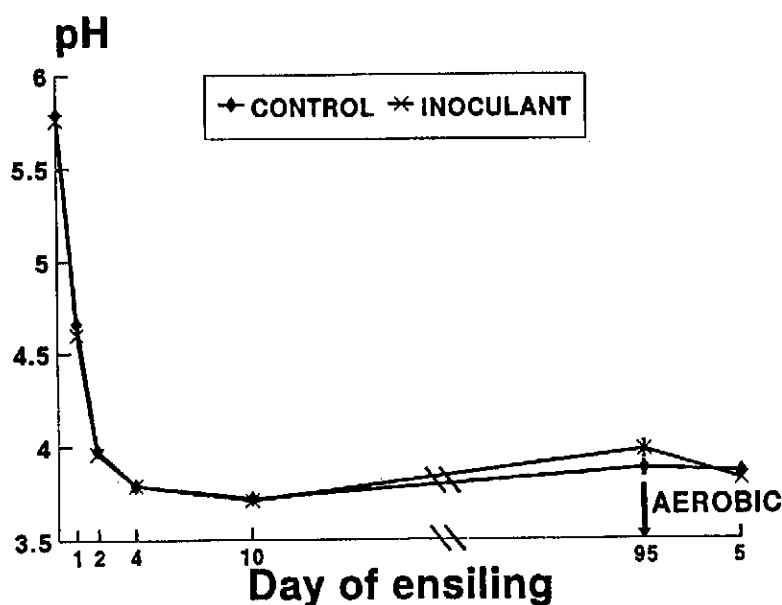


Figure 1. The change in pH of maize silage ensiled with or without a lactic acid bacterial inoculant. SEM = 0.02. (Standard error bars are shown where significant ($P < 0.05$) differences were found)

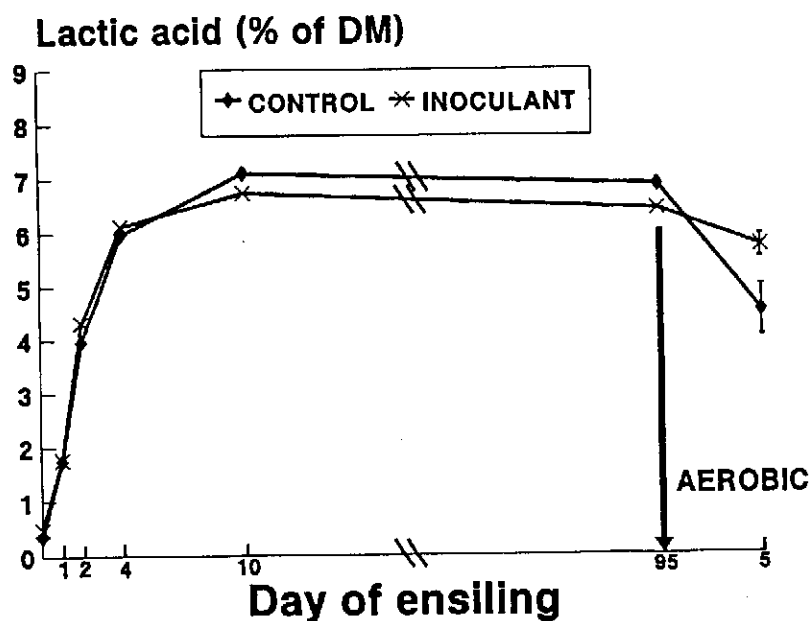


Figure 2. The change in lactic acid of maize silage ensiled with or without a lactic acid bacterial inoculant. SEM = 0.34 (Standard error bars are shown where significant differences were found).

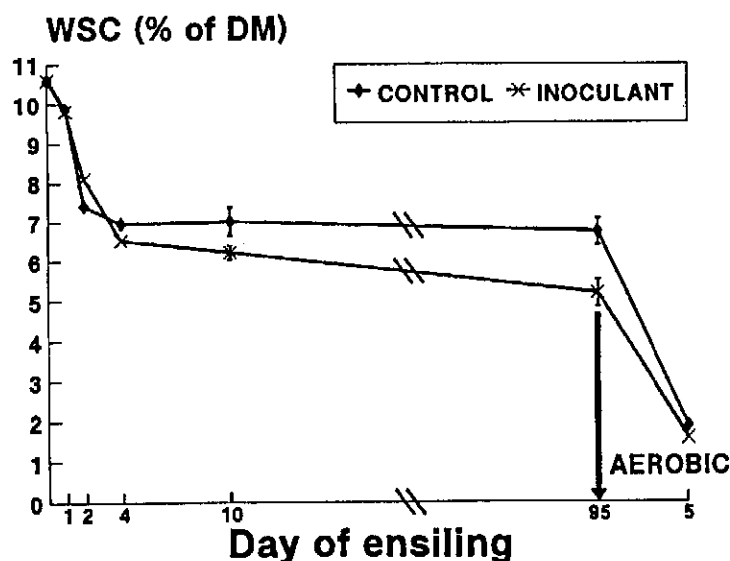


Figure 3. The change in water soluble carbohydrate of maize silage ensiled with or without a lactic acid bacterial inoculant. SEM = 0.53 (Standard error bars are shown where significant differences were found).

Table 1

Composition (% of DM) of maize silage made with or without the addition of a lactic acid bacterial inoculant

	Control	Inoculant	SEM ^a
Dry matter	27.6	27.6	0.20
Organic matter	89.5	88.8	0.20
NDF	49.6	49.2	0.50
IVOMD	74.5	75.2	0.40
Crude protein	9.3	9.4	0.12
NH ₃ -N(% of TN)	5.3	5.2	0.10
pH	3.7 ^b	3.9 ^c	0.01
WSC ^d	7.1 ^b	5.2 ^c	0.13
Lactic acid	6.9 ^b	6.4 ^c	0.06
Acetic acid	1.1 ^b	1.4 ^c	0.03
N-Butyric acid	NF ^e	NF	
Iso-Butyric acid	NF	NF	
Propionic acid	NF	NF	
Isovaleric acid	NF	NF	
n-Valeric acid	NF	NF	

^aSEM = Standard error of means; ^{b,c}Means with different superscripts in the same row differ significantly (P < 0.05); ^dWSC = Water soluble carbohydrates; ^eNF = Not found.

Table 2

Microbial examination (log CFU g⁻¹ fresh material) of maize ensiled with or without a lactic acid bacterial inoculant.

Treatment	Day of ensiling	LAB ^a	E ^b	Y ^c	M ^d	Cl ^e
<i>Control</i>						
Fresh material	0	9.3	5.1	2.6	2.0	3.0
Silage	1	> 10.5	4.5	2.6	2.1	2.5
	2	> 10.5	4.5	2.3	2.0	2.5
	4	> 10.5	4.0	2.0	2.0	0.9
	10	> 10.5	3.9	1.9	1.9	0.9
	95	7.6	2.1	2.1	NF ^f	0.6
Aerobic	5	> 10.5	2.1	2.0	NF	0.3
<i>Inoculant</i>						
Fresh material	0	9.5	4.5	2.3	2.3	0.6
Silage	1	> 10.5	4.4	2.0	2.1	0.3
	2	> 10.5	4.0	2.0	2.0	0.3
	4	> 10.5	4.0	1.9	1.9	NF
	10	> 10.5	3.9	1.7	1.8	NF
	95	7.6	4.6	2.6	2.0	NF
Aerobic	5	> 10.5	4.5	2.1	1.9	NF

^aLAB = Lactic acid bacteria; ^bE = Enterobacteria; ^cY = Yeast; ^dM = Mould;

^eCL = Clostridial spores; ^fNF = Not found.

Both silages were unstable when exposed to air for 5 days as indicated by production of 9.9 and 7.6 g CO₂ kg⁻¹ DM of control and inoculated silage, respectively. The drop in lactic acid (Fig. 2) and WSC (Fig. 3) during the aerobic exposure also indicated deterioration of the silage. The minimum and maximum temperature during the aerobic stability test was 11 and 23°C, respectively.

3.2. Growth study

The yield of plant material was 78.7 tonne of wet or 23.1 tonne of dry matter per hectare. The dry matter content of the control silage and the inoculated silage made in the 210 litre drums was

28.6% and 29.3%, respectively. The pH was 3.5 for both silages. The IVOMD and crude protein content was 70.0% and 9.0% for the control and 69.5% and 8.8% for the inoculated silage, respectively. No butyric acid was detected in either silages. The control and the inoculated silage contained 3.1 and 3.4% acetic acid, respectively. The lactic acid content was 5.8 and 5.4% and the WSC content was 2.2 and 2.3% for the control and inoculated silage, respectively.

Table 3

Performance of lambs fed control and inoculated maize silage diets

	Control	Inoculant	SEM ^a	P-value ^b
<i>Weight of lambs (kg)</i>				
Day 0	26.4	26.4	0.46	1
Day 30	33.1	33.4	0.46	0.22
Day 60	39.8	41.1	0.49	0.08
<i>Average daily gain (g)</i>				
Day 0 to 30	267	289	15	0.34
Day 0 to 60	239	255	10	0.26
<i>Silage intake (g DM day⁻¹)</i>				
Day 0 to 30	568	588	22	0.54
Day 0 to 60	708	784	32	0.11
<i>Total intake (g DM day⁻¹)</i>				
Day 0 to 30	1028	1048	22	0.54
Day 0 to 60	1118	1244	48	0.07
<i>Feed conversion ratio (kg DM feed kg⁻¹ LWG^c)</i>				
Day 0 to 30	4.00	3.74	0.2	0.37
Day 0 to 60	4.94	4.93	0.16	0.97

^a SEM = Standard error of means; ^b P-value = Level of significance; ^c LWG = Liveweight gain.

The dry matter intake of the total diet by lambs over the growth period was $3.21 \pm 0.13\%$ and $3.23 \pm 0.11\%$ of liveweight for the control and the inoculant treatment, respectively. The

performance of lambs fed the control and inoculated maize silage diets is given in Table 3. The silage constituted $58.0 \pm 2.8\%$ and $60.2 \pm 3.7\%$ of the total dry matter intake during the growth period for the control and inoculant treatment, respectively. The average daily gain of lambs over the 60 days in the feedlot was 239 ± 26 and 255 ± 44 g day⁻¹ for the control and the inoculant treatment, respectively. This difference was not statistically significant ($P = 0.26$).

The adding of the lactic acid bacterial inoculant tended to result in a higher intake of silage ($P = 0.11$) over the sixty days of the growth trail (Table 3) compared to the control treatment. Liveweight of lambs fed inoculated silage tended ($P = 0.08$) to be 3.2% higher than that of lambs fed the control diet after the 60 days growth period. This resulted in a shortening of the finishing period by 5 days when inoculated maize silage (60 days) was fed compared to control silage (65 days). The feed conversion ratio did not differ between the two diets (Table 3).

4. Discussion

The adding of the lactic acid bacterial inoculant to maize crop at ensiling did not result in a more rapid drop in pH and a higher amount of lactic acid production compared to the control treatment as found by Meeske *et al.* (1993) when forage sorghum was ensiled with lactic acid bacterial inoculants. The lack of response can be attributed to the high numbers of lactic acid bacteria which were present on the maize crop prior to ensiling (Table 2) on the control treatment. Although higher numbers of clostridial spores were detected on the control maize silage compared to the inoculated silage (Table 2) it did not result in more breakdown of protein in the control silage. No butyric acid was detected in any of the silages which could be explained by the poor correlation between spore numbers and the products of clostridial fermentation, such as butyric acid and ammonia (McDonald *et al.*, 1991).

The IVOMD and crude protein (CP) of maize silage found in the present study (Table 1) is comparable to the $74 \pm 3\%$ IVOMD and $7.7 \pm 0.8\%$ CP found by Aufrère *et al.* (1992) on 118 whole maize plant samples. Maize silage is an excellent feed source for growing lambs as was clearly shown by the growth rate of 255 g day⁻¹ on a diet containing 60% inoculated maize silage.

The inoculated maize silage was more palatable as was reflected by a tendency for higher intake (Table 3) compared to the control silage. The average daily gain of the lambs fed inoculated maize silage was not significantly ($P < 0.05$) higher than that of lambs fed the control silage. The feed conversion efficiency of lambs was not affected by the inoculation of maize silage as was also found by Scheafer *et al.* (1989). Differences in intake of silage and growth of lambs could possibly have been more pronounced between treatments if less concentrate was fed. The concentrate was fed at 40% of the total diet to obtain a satisfactory growth rate under feedlot conditions. The rate of drop in pH for both the control and inoculated silages was more rapid in the present study compared to that found by Rust *et al.* (1989). Rust *et al.* (1989) found that inoculating maize silage with a microbial inoculant did not affect animal weight gains and dry matter intake, but the inoculant appeared to lower the stability of maize silage to air exposure. In the present study the adding of inoculant did not have a detrimental effect on the aerobic stability of maize silage.

Although the laboratory study showed very little effect of adding a lactic acid bacterial inoculant to maize at ensiling, there was a definite tendency for lambs to consume more of the inoculated silage and grow at a faster rate. This resulted in a shortening of the fattening period by 5 days and a saving of 2.5 kg of concentrate and 5 kg of silage per lamb fed the inoculated silage.

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The effect of the addition of a lactic acid bacterial inoculant with an amylolytic enzyme to maize at ensiling on silage composition, silage intake, milk production and milk composition.

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Abstract

The aim of the study was to determine the effect of the addition of a lactic acid bacterial inoculant with an enzyme to maize at ensiling on the fermentation dynamics during ensiling, aerobic stability of the silage as well as the intake, milk production and milk composition of Jersey cows fed maize silage diets. Maize silage was made in laboratory silos to determine the effect of the addition of an inoculant on fermentation dynamics during ensiling. The inoculant contained *Lactobacillus plantarum* and *Pediococcus acidilactici* as well as amylase. Twelve silos were filled with each of inoculated and untreated chopped maize plants and three silos of each treatment were opened on each of days 2, 10, 50 and 90 of ensiling. One bunker silo was filled for each of untreated and inoculated maize silage for the intake and milk production study. The inoculant did not result in a more rapid lowering of the pH or a more rapid lactic acid production compared to untreated maize silage made in laboratory silos. Both the control and inoculated maize silages were well preserved with a pH of 3.57 and 3.62, a lactic acid content of 66 and 63 g/kg DM and an ammonia nitrogen content of 5.88 and 5.10 g/100 g of total nitrogen respectively. No butyric acid was found in either untreated or inoculated maize silage. The maize silages made in the bunker silos were well preserved with a DM of 283 and 307 g/kg silage, pH of 3.5 and 3.51, lactic acid of 37.0 and 35.3 g/kg DM for the control and inoculated maize silage, respectively. No butyric acid was found in any of the silages. The addition of the inoculant to maize at ensiling

improved the palatability, intake and the aerobic stability of maize silage compared to the untreated control maize silage. The intake of untreated and inoculated maize silage by Jersey cows was 7.6 and 8.4 kg DM/day for the control and inoculant treatment, respectively. Milk production, milk composition, liveweight and condition score of Jersey cows was not significantly affected by the addition of the inoculant to maize silage. The aerobic stability of maize silage was improved by the addition of the inoculant.

Keywords: Maize silage, inoculant, lactic acid bacteria, amylolytic enzyme, milk production, intake, Jersey cows.

1. Introduction

Maize (*Zea mays*) is an ideal silage crop with a relative high dry matter (DM) content, low buffering capacity and adequate water soluble carbohydrates for satisfactory fermentation to lactic acid (McDonald *et al.*, 1991). To obtain a high quality well fermented palatable silage, a rapid drop in pH is needed to inhibit the growth of enterobacteria and clostridia (McDonald *et al.*, 1991). This happens when homofermentative lactic acid bacteria utilise water soluble carbohydrates and produce lactic acid. If, however heterofermentative lactic acid bacteria are dominant on a maize crop prior to ensiling fermentation will be less efficient and the end products of fermentation will be lactic acid, acetic acid, ethanol and carbon dioxide (McDonald *et al.*, 1991). Meeske and Basson (1998) found that the number of lactic acid bacteria on fresh chopped maize plants prior to ensiling was as high as 10^9 colony forming units per gram of fresh material. This study was done at the Animal Nutrition and Animal Products Institute, Irene (longitude $28^{\circ} 13'S$: latitude $25^{\circ} 55'E$, altitude 1524m). Weise and Wermke (1973) established that lactic acid bacteria prefer moderately warm weather. Lower numbers of lactic acid bacteria may therefore be expected on silage crops in more temperate climates as is found in the Southern Cape (longitude $22^{\circ} 25'S$: latitude $33^{\circ} 55'E$, altitude 204m). Speckman *et al.* (1981) surveyed numbers of lactobacilli on maize crops in the USA and showed that 69 % of samples had counts below 1000 colony forming units per gram. The aim of this study was to determine the effect of adding a lactic acid bacterial inoculant with an amylolytic enzyme to maize at the time of ensiling on

fermentation dynamics during ensiling, aerobic stability of the silage, intake, milk production and milk composition of Jersey cows fed maize silage diets.

2. Materials and methods

Four hectares of maize (PAN 6364) was planted on the 10th of December 1997 at the Outeniqua Experimental Farm (longitude 22° 25 'S : latitude 33° 55'E, altitude 204m). The maize was harvested at the half to three quarter milk line at a dry matter content of 30% on the 18th of March 1998. Whole crop maize was chopped with a PZ Zweegers WH90S silage chopper.

2.1 Laboratory study

A laboratory study was done to determine the effect of an inoculant on the fermentation dynamics of maize during ensiling. Forty kilogram of chopped maize was mixed on a polyethylene surface which was cleaned with ethanol. The material was then divided into two portions of 20 kg. The lactic acid bacterial (LAB) inoculant Maize-all (Supplied by Alltech Biotechnology Pty. Ltd.) contained *Lactobacillus plantarum* and *Pediococcus acidilactici* as well as amylase. The inoculant was applied at 5 g tonne⁻¹ of fresh material to provide 10⁶ Colony forming units of lactic acid bacteria per gram of fresh material. Maize was ensiled in 1.5 litre Weck glass jars (J. WECK, GmbH u. Co., Wehr-Oflingen, W. Germany) with glass lids, fastened with metal clamps which enables gas release. Twelve silos were filled with each of inoculated and untreated chopped maize plants. Three silos of each treatment were opened on each of days 2, 10, 50 and 90 of ensiling and representative samples were taken for chemical (stored at -20°C) and microbiological analysis. At day 90 of ensiling, silage was exposed to air to determine the aerobic stability. Silage was put in three 2 litre polyethylene terephthalate bottles for each treatment as described by Ashbell *et al.* (1991). Two bottles were filled with wheat straw to monitor the room temperature. All bottles were fitted with a T-type thermocouple and placed in a polystyrene container in a room kept at a temperature between 20 and 25°C. Temperature changes were measured at hourly intervals using a MC- System 120-02EX, 16 canal data logger for a period of ten days.

Microbiological analyses were carried out on a representative sample of the three replicates for each of the control and treated silage for each of the test days. Microbial analyses on fresh plant material before ensiling, was done after the additive was applied. Forty grams of material was weighed into sterile stomacher bags, 360 ml of sterile saline water added and the samples were homogenized by stomaching for 3 minutes. The extract was further diluted. Enumeration of lactobacilli was done using MRS agar according Oxoid (1990) and lactococci was determined using M17. Colonies were counted directly on the agar plates. Agar plates were incubated at 37°C for 72 hours. Yeasts were enumerated according to the IDF standard 94B procedure, 1990. Plates were incubated at 25°C for 72 hours.

Dry matter of the fresh material and silage was estimated by drying samples in an oven at 60°C for 72 hours. Water soluble carbohydrates (WSC), pH and lactic acid were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 minutes with a stomacher. WSC were determined according to Marais (1979) and LA was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acids (VFA) were determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35m x 3mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H_3PO_4). The column was conditioned for 48 hours at 165°C with a nitrogen carrier gas flow of 40 ml per minute. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963). Total nitrogen was determined by the Kjeldahl method (AOAC, 1984). The ammonia nitrogen content of silage was determined by homogenizing 50g of silage in 250ml of a 0.1N H_2SO_4 solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Mettöhm 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen. Least significant differences between treatments in the laboratory study were determined by a one-way ANOVA, using the Statgraphics (1988) statistical computer programme.

2.2 Intake and milk production study

The inoculant was applied on the silage chopper with an applicator to provide 10^6 colony forming

units (CFU) of lactic acid bacteria per gram of fresh material. One bunker of 4m x 1.2m x 25m was filled with control and one bunker with inoculated silage within a period of four days (2 days/bunker). Silage was stored for a period of eight months. Aerobic stability of silage was determined two times with 10 day intervals during each of the two feed out periods as described for the laboratory study. Representative control and inoculated maize silage samples were taken directly after the silage was removed from the bunker.

Twenty two multiparous cows averaging 127 days in milk were blocked in pairs according to milk production (previous 4 weeks), days in milk, lactation number, liveweight and condition score. Within each block, cows were randomly allocated to either control or inoculated silage treatment. The control and inoculated maize silage diets were fed to the two groups of cows for two periods of 30 days in a two by two cross over design. Each period consisted of a 10 day adaption and 20 days measurement period. Milk production was recorded daily and milk composition weekly. A composite sample of afternoon and morning milking was taken for determination of protein, butterfat, lactose and milk urea nitrogen. Liveweight and condition score of cows were determined on two consecutive days after milking at 09h00 on day 0, 30 and 60 of the experimental period.

Samples of control silage, inoculated maize silage and concentrates were taken on Mondays, Wednesdays and Fridays and were frozen at -4°C. Samples were pooled for each week of the experimental period. This resulted in three composite samples for each of the control silage, inoculated silage and concentrate for each period. Samples of maize silage were processed and DM, OM, IVOMD, crude protein (CP), pH, lactic acid, acetic acid, propionic acid and butyric acid were determined as described for the laboratory study. Neutral detergent fibre (NDF) was determined according to Van Soest *et al.* (1991), water soluble carbohydrates according to Marais (1979) and starch according to Rasmussen and Henry (1990).

Cows were milked two times per day at 06:00 and 15:30 and each cow received 5.5kg DM of concentrate daily. The concentrate was divided into two equal portions and was fed after each milking. The dairy concentrate consisted of 34.2% maize, 15% wheat, 5% molasses meal, 6% fishmeal, 5% wheat bran, 12% cottonseed, 18% cottonseed oilcake, 2% feed lime, 0.5% dicalciumphosphate, 1% salt, 1% urea, 0.3% mineral premix on a dry matter basis. The concentrate was formulated to contain 26% crude protein, 12 MJ ME/kg, 1.1% Ca and 0.65% P on a dry

matter basis. Dry matter intake of silage was determined on a daily basis. Silage was fed individually to cows and cows had free access to silage from 8:00 to 12:00 and from 16:30 to 20:30. Cows were kept in a small rest camp with access to water only from 12:00 to 15:30 and from 20:30 to 06:00 the next morning.

Data of the milk production study were analysed using SAS (1996). The GLM procedure was followed for a cross-over design with two treatments and two periods.

3. Results

The changes in pH, WSC and lactic acid in the control and inoculated silage during the ensiling period and after 10 days of aerobic exposure at the end of the ensiling period is given in Table 1.

Table 1

The chemical composition (g/kg DM) and microbial analysis of maize ensiled in laboratory silos for 90 days with or without the addition of a lactic acid bacterial inoculant.

	Control	Inoculant	SEM ^c
n	3	3	
Dry matter	263 ^a	253 ^b	1.0
Organic matter	953	952	1.8
<i>In vitro</i> digestible organic matter	718	724	8.7
Crude protein	82 ^a	76 ^b	0.5
NH ₃ -N (% of Total nitrogen)	5.88 ^a	5.10 ^b	0.03
Acetic acid	0.08	0.06	0.07
Propionic acid	NF ^d	NF	
N-Butyric acid	NF	NF	
Gas loss g/ 100gDM	6.47	6.25	0.18
Microbial analysis (log ₁₀ CFU/g silage)			
Yeast	1.34	0.82	0.91
Lactobacilli	4.88	5.02	4.03
Lactococci	5.41 ^a	7.94 ^b	6.28

^{ab}Means with different superscripts in the same row differ significantly ($P < 0.05$)

^c Standard error of mean, ^d Not found

Untreated and inoculated silages were stable when exposed to air and no increase in temperature above the ambient temperature was recorded over a period of 240 hours. The pH, WSC and lactic acid content of untreated and inoculated maize silage did not change during aerobic exposure (Table 1).

The chemical composition of maize ensiled in laboratory silos for 90 days is given in Table 2.

Table 2

The pH, water soluble carbohydrate and lactic acid content (g/kg DM) of untreated and inoculated maize silage after 2, 10, 50, 90 days of ensiling and after 10 days of aerobic exposure.

	Day 0	Day 2	Day 10	Day 50	Day 90	Day 10 Aerobic
n	3	3	3	3	3	3
pH						
Control	5.75	3.90	3.71 ^a	3.71 ^a	3.57	3.63
Inoculant	5.75	3.85	3.68 ^b	3.69 ^b	3.61	3.62
SEM ^c	0.06	0.02	0.01	0.003	0.01	0.02
WSC ^d						
Control	159	100	64	19 ^a	35	41
Inoculant	159	106	55	25 ^b	32	34
SEM	11	14	12	1.5	5.6	3.8
Lactic acid						
Control	3.4	34	50	51	71	66
Inoculant	3.4	36	52	61	68	63
SEM	1.3	1.1	2.9	3.2	2.3	3.0

^{a,b}Means with different superscripts in the same column differ significantly ($P < 0.05$)

^c Standard error of mean.

^d Water soluble carbohydrates

No butyric acid was found in either the control or in the inoculated silage and the ammonia nitrogen as percentage of total nitrogen was low in both silages. This indicates that both silages

were well preserved.

The chemical composition of maize silage made in bunker silos is given in Table 3.

Silages were well preserved as indicated by the low pH and absence of butyric acid.

The IVOMD of the concentrate was $80.9 \pm 1.2\%$, the crude protein 262 ± 13 g/kg DM, the NDF 132 ± 22 g/kg DM, the calcium 20.1 ± 9 g/kg DM and the phosphorous content 6.6 ± 2 g/kg DM.

Table 3

The chemical composition (g/kg DM) of bunker maize silage with or without the adding of a lactic acid bacterial inoculant.

	Control	Inoculant	P-value	SEM ^c
n	6	6		
Dry matter	283 ^a	307 ^b	0.01	4.6
Organic matter	957	959	0.25	0.13
In vitro organic matter digestibility	705	710	0.72	10
Crude protein	78.4 ^a	83.0 ^b	0.002	0.8
Ammonia-N /100g of Total nitrogen	8.16 ^a	5.29 ^b	0.000	0.23
Water soluble carbohydrates	40 ^a	54 ^b	0.0002	1.7
Neutral Detergent Fibre	467	439	0.12	12
Starch	233	259	0.14	11.7
pH	3.50	3.51	0.78	0.04
Lactic acid	37.9	35.3	0.47	2.5
Acetic acid	27.0 ^a	20.1 ^b	0.01	1.4
Propionic acid	1.4	1.7	0.74	0.8
N-Butyric acid	NF ^d	NF		

^{ab}Means with different superscripts in the same row differ significantly ($P < 0.05$)

^c Standard error of mean.

^d Not found

The intake, milk production and milk composition of Jersey cows fed control or inoculated maize silage diets is given in Table 4.

Table 4

Intake, milk production and milk composition of Jersey cows fed control or inoculated maize silage diets.

	Control	Inoculant	P-value	SEM ^c
Milk production (kg/day)	15.6	15.9	0.47	0.36
Fat corrected milk production (kg/day)	17.4	17.5	0.75	0.40
Butterfat %	4.79	4.74	0.55	0.08
Protein %	3.52	3.55	0.36	0.02
Lactose %	4.89	4.86	0.22	0.02
Milk urea nitrogen mg/dl	11.4	11.8	0.40	0.32
Dry matter intake (kg/day)				
Silage	7.6 ^a	8.4 ^b	0.0003	0.13
Concentrate	5.5	5.5		
Total	13.1 ^a	13.9 ^b	0.0003	0.13
% of Liveweight	3.78 ^a	3.99 ^b	0.001	0.04
Change in liveweight (g/day)	0.31	0.34	0.61	0.04
Change in condition score	0.17	0.13	0.56	0.11

^{ab}Means with different superscripts in the same row differ significantly ($P < 0.05$)

^c Standard error of mean.

During period 2, one cow that was fed the control silage got mastitis resulting in a very low intake and milk production. Data of the affected cow, as well as that of the cow with which she was blocked, were removed for both periods

The aerobic stability of control and inoculated bunker maize silage over a period of sixty hours is given in Figure 1.

The control and inoculated bunker maize silages were stable for 7 and 39 hours, 28 and 28 hours, 8 and 19 hours and 4 and 7 hours during aerobic exposure test 1, 2, 3 and 4, respectively.

Figure 1. Changes in temperature for control and inoculated maize silage during 60 hours of aerobic exposure determined 4 times during the feed out period.

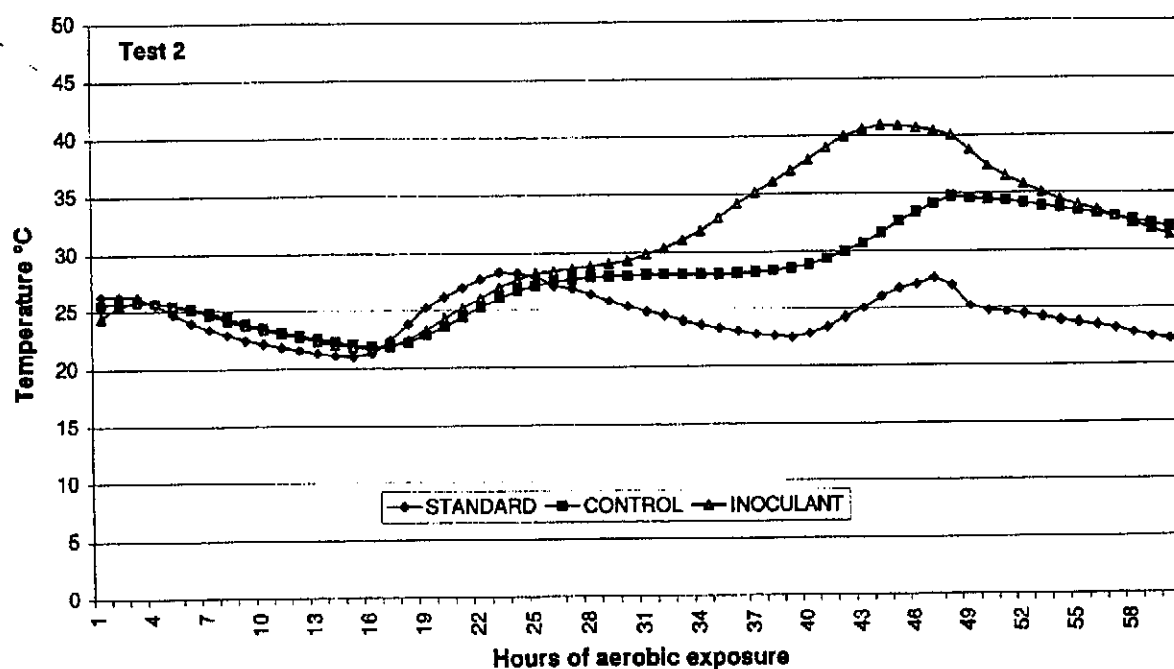
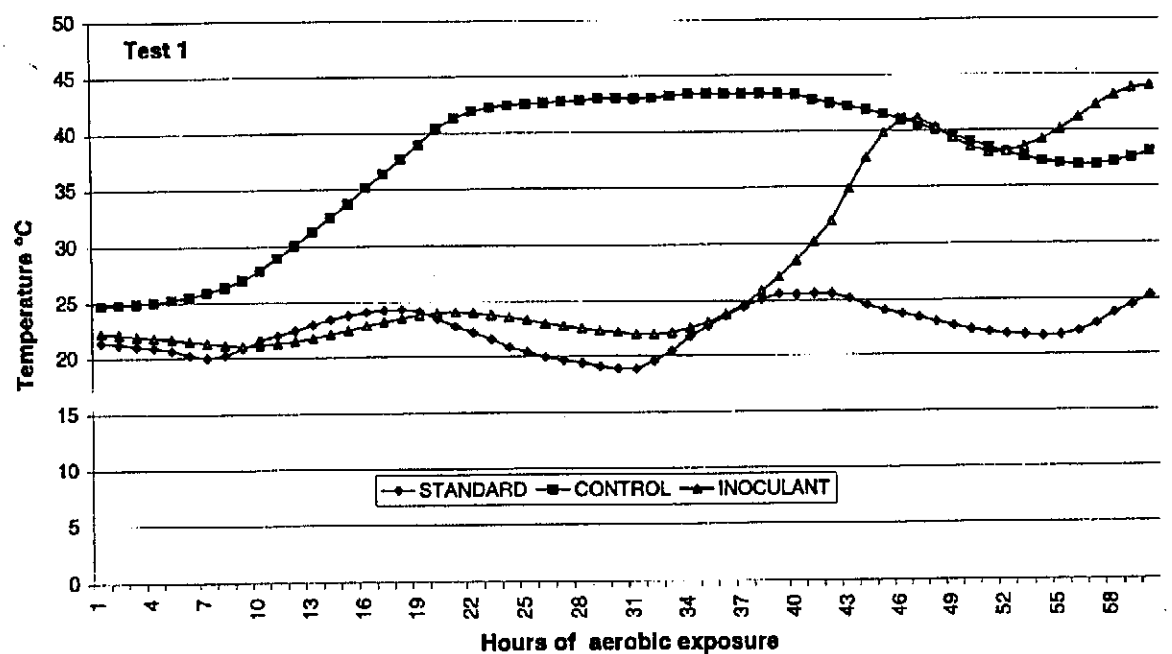
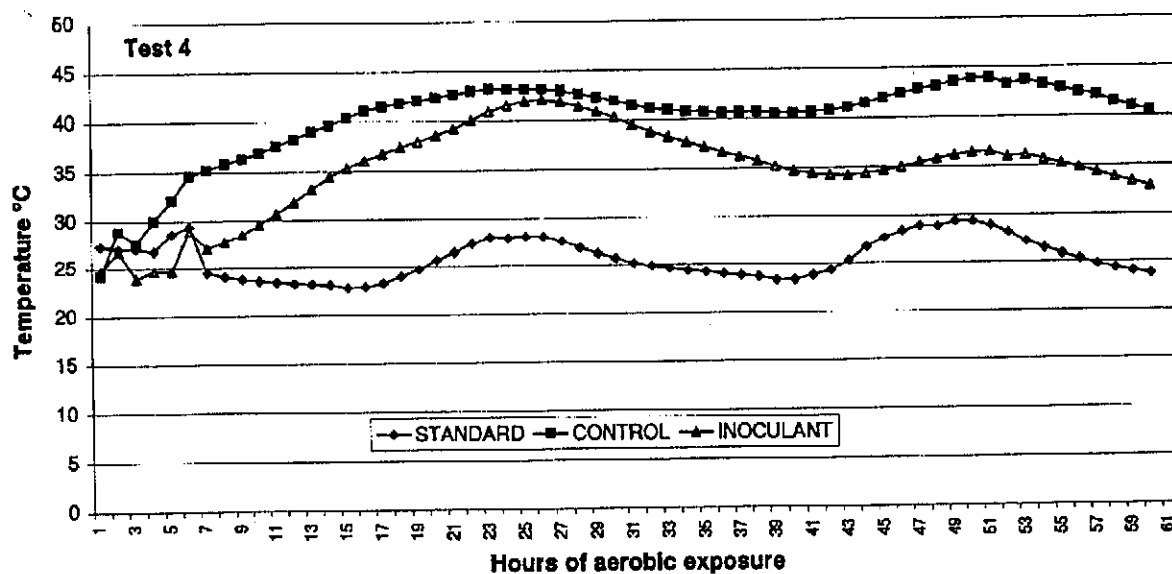
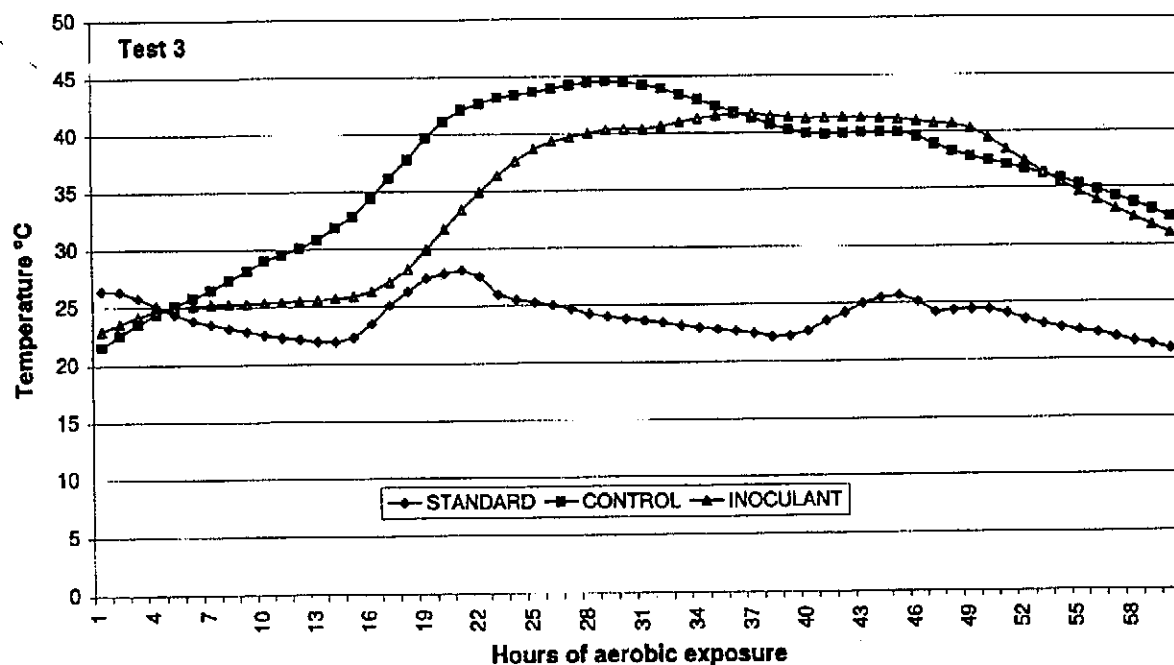


Figure 1(continued). Changes in temperature for control and inoculated maize silage during 60 hours of aerobic exposure determined 4 times during the feed out period.



4. Discussion

4.1 Laboratory study

Both the control and inoculated maize silages were well preserved as indicated by the low pH, high lactic acid content, low level of ammonia-nitrogen and absence of butyric acid. The pH drop in maize silage was much more rapid in our study with the pH at 3.9 and 3.85 for control and inoculated silage after only two days of ensiling compared to the pH of 4.5 after 5 days of ensiling as was found by Rust *et al.* (1989). Meeske and Basson (1998) also found that the pH of maize silage dropped to 3.99 after two days of ensiling. The inoculated maize silage in our study had a lower pH than the control silage on day 10 and 50 of ensiling (Table 1) but no differences were found after 90 days of ensiling. The lactic acid content of maize silage was not affected by the addition of the inoculant. The level of lactic acid in maize silage (Table 1) compares well with the 63 to 120 g lactic acid/kg DM found by Spoelstra and Van Wikselaar (1992) in maize silage made in laboratory silos.

The crude protein (CP) content was lower in the inoculated silage than in the control, suggesting a higher rate of protein breakdown or nitrogen loss in this treatment. The reason for this is not apparent. The ammonia-nitrogen concentration (per 100 gram of total N) in the inoculated maize silage was also lower than in the control maize silage. Looking at $\text{NH}_3\text{-N}$ values alone, one might be tempted to reason that the lower value would suggest less protein breakdown, but with the CP content being lower in the inoculated silage, this observation cannot be explained by the degree of protein breakdown. With the higher moisture content of the control treatment, it might have been possible that more $\text{NH}_3\text{-N}$ stayed soluble in the control, while a larger proportion escaped into the atmosphere in the case of the inoculated silage, hence the lower $\text{NH}_3\text{-N}$ content of the analysed material. If this was the case, then the apparent nitrogen loss from NH_3 escaping into the atmosphere could account for about half of the difference observed in CP content between the two silages. Whatever the reason, the $\text{NH}_3\text{-N}$ content was at an acceptable low level in both treatments, and the difference between the treatments is of no practical importance.

The addition of the inoculant to maize did not affect the IVOMD.

The number of Lactococci were significantly higher in the inoculated maize silage compared to the untreated control silage. This may be as a result of the added *Pediococcus acidilactici* bacteria present in the inoculant as Pediococci are often found in very low numbers on cereal crops (Woolford, 1984). The number of lactobacilli did not differ between control and inoculated maize silage after 90 days of ensiling. The yeast counts on both the control and inoculated maize silage was low compared to the 2.6 log CFU/g of fresh material found by Meeske and Basson (1998) on maize silage after 90 days of ensiling. The low yeast counts may be a result of the rapid lowering of the pH in the silage as well as the rapid exclusion of air that occurs in laboratory silos (McDonald *et al.*, 1991).

The water soluble carbohydrate content of maize prior to ensiling was high at 159g/kg DM. The residual WSC after 90 days of ensiling would indicate that sufficient nutrients were available for the lactic acid bacteria to grow. Addition of the inoculant did not have any affect on the WSC levels during fermentation, indicating that WSC was utilized at the same rate in the untreated and inoculated maize silages. This is in contrast with the more rapid WSC utilization found by Meeske *et al.* (1999) when adding an inoculant to tropical grass silage.

4.2 Intake and milk production study

The inoculated and control maize silages made in bunker silos were both well preserved (Table 3). The DM content of the maize silages of 28 to 30% (Table 3) were optimal to ensure maximum dry matter intake (Phipps and Wilkinson, 1985). The inoculated silage had a higher DM, non structural carbohydrate and CP content and a lower acetic acid content compared to that of the control silage. This may indicate a more efficient fermentation. The level of acetic acid of 27g/kg DM and 20g/kg DM found in the control and inoculated maize silage respectively was high compared to the 6g/kg DM found by Scheafer *et al.* (1989) in bunker maize silage. Ashbell and Lisker (1988), however, reported acetic acid levels of 15 to 21g/kg DM in maize silage made under farm conditions in a subtropical climate, while Spoelstra and Van Wikselaar (1992) found

levels of 10 to 21g acetic acid/kg DM.

The starch content of the inoculated and enzyme treated maize silage did not differ from that of the control maize silage (Table 3). This indicates that the amylase did not breakdown starch as was found by Spoelstra and Van Wikselaar (1992) where the starch content of maize silage was reduced by up to 50% when enzymes with amylolytic activity were added to maize at ensiling. The WSC content of the inoculated maize silage was higher and acetic acid content lower than that of the control maize silage. This indicates that inoculation did result in more efficient homofermentative fermentation. The NDF of the inoculated maize silage tended ($P=0.12$) to be lower than that of the control maize silage. This may be as a result of more hydrolysis of hemicellulose in the inoculated maize silage (McDonald *et al.*, 1991). Hemicellulose may be broken down during ensiling by hemicellulases present in the original herbage, bacterial hemicellulases and hydrolysis by organic acids produced during fermentation (McDonald *et al.*, 1991).

The CP content of the inoculated silage was higher ($P<0.05$) than that of the control silage. Less protein breakdown occurred in the inoculated maize silage compared to the control maize silage as indicated by the ammonia nitrogen as percentage of total nitrogen presented in Table 4 was lower than that of the control silage, indicating less protein breakdown in the former. This is in contrast with the results found in the laboratory study. The protein content of the maize silage was similar to that reported by Cilliers *et al.* (1998) and was higher than the $69\pm4\text{g/kg DM}$ reported by Meeske *et al.* (2000) on twenty one maize hybrids.

The milk production, fat corrected milk production and milk composition of cows did not differ significantly between the control and inoculated silage diets (Table 4). The intake of inoculated silage was significantly ($P<0.05$) higher than that of the control silage (Table 4). Silage intake of cows receiving the inoculated silage in Period 1 was 8.68 kg DM/day and this decreased to 7.49 kg DM/day during Period 2 when control silage was fed. The inoculated silage appeared to be more palatable than the control silage. This may have been caused by the lower acetic acid content and improved aerobic stability of the inoculated maize silage compared to that of the

control maize silage. Similar observations were reported by Meeske *et al.* (1999) who found that inoculated tropical grass silage had a lower acetic acid content and a higher intake than that of untreated silage. Meeske and Basson (1998) also found that, although the chemical composition of untreated and inoculated maize silage was similar, lambs tended ($P=0.07$) to ingest more of the inoculated silage than of the control silage. Honig and Daenicke (1993) found that Simmental bulls consumed more of an inoculated maize silage than an compared to untreated control silage, although no marked improvement in silage fermentation was found. Rust *et al.* (1989) added a lactic acid bacterial inoculant at 2×10^5 CFU per gram of fresh material to maize and increased the lactobacillus organisms on the crop by 15% at the time of ensiling. This resulted in an increased lactic acid content in the inoculated maize silage but had no effect on intake and weight gains by crossbred steers.

The inoculant had no effect on changes in liveweight and condition score of cows over a 60 day period.

Aerobic deterioration of maize silage is initiated by yeasts or acetic acid bacteria (Driehuis and Van Wixselaar, 1996). The inoculated maize silage in our study was more stable than the control maize silage as is shown in Figure 1. This is in agreement with the study of Woolford (1975) and differs from the work of Moon *et al.* (1980) and Rust *et al.* (1989), who found that inoculated maize silage was less stable than untreated maize silage when exposed to air. Spoelstra and Van Wixselaar (1992) showed that the starch in maize silage is degraded relatively easy by amylase. The liberated sugars are then fermented to ethanol by yeasts, resulting in higher yeast counts and lowered aerobic stability of enzyme treated maize silage. Scheafer *et al.* (1989) and Sanderson (1993) found that resistance to aerobic deterioration was not affected by the use of a lactic acid bacterial inoculant on maize silage.

The number of yeast present on the crop at ensiling as well as the time from harvesting until anaerobic conditions prevail has a major impact on the aerobic stability of maize silage. Our results show that a large variation in aerobic stability of maize silage occurred. Conditions when making the laboratory maize silage were ideal resulting in both the control and inoculated silages

being stable when exposed to air. Bunker maize silage on the other hand, which was made under less favourable conditions, was more susceptible to aerobic deterioration. Therefore, when evaluating the effect of additives on the aerobic stability of silage, laboratory studies should be followed up with large scale studies where silage is made on a commercial scale.

5. Conclusions

Maize ensiled in laboratory silos differed markedly from maize ensiled in bunker silos. The laboratory silos created optimal ensiling conditions. This resulted in maize silage with a high lactic acid content, low acetic acid content and a high aerobic stability. Maize silage made in bunker silos had a lower lactic acid content and a higher acetic acid content than that of maize silage made in laboratory silos. The inoculant improved the aerobic stability of maize silage made in a bunker compared to untreated maize silage. Studies to determine the effect of additives on the aerobic stability of silages done with laboratory silos should be followed up with studies on large scale commercial silos.

The addition of the inoculant did not affect fermentation dynamics of maize during the ensiling period or the chemical composition of maize silage to a large extent. The addition of the lactic acid bacterial inoculant did improve the palatability of maize silage and resulted in a significantly ($P<0.05$) higher intake of silage by Jersey cows. Milk production, milk composition, liveweight change and condition score of cows fed inoculated maize silage was not significantly different from that of cows fed control maize silage.

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Chapter 6

The ensiling of oats

The effect of adding an enzyme containing lactic acid bacterial inoculant to big round bale oat silage on intake, milk production and milk composition of Jersey cows.

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Introduction

Small grain cereals can be grown in a wide range of climatic and soil conditions (Kennelly and Khorasani, 1999). In the Western Cape oats is one of the most popular silage crops. It is used in rotation with wheat planted for grain. Oats is often chopped at the bloom stage with a flail harvester and ensiled in bunker silos. During the last three years many farmers have however changed to big round bale silage. The main reason for this is the flexibility and convenience of making and handling big round bale silage. Givens *et al.* (1993) pointed out that big bales have a lower density and a larger surface area relative to mass than bunker silage. The risk of aerobic deterioration is therefore high for silages in badly sealed or damaged wrapped bales. Fenlon *et al.* (1989) warns that big bale silage is prone to contamination with listeria organisms. The degree of listeria contamination can be largely reduced by the removal of spoiled material prior to feeding.

Khorasani *et al.* (1997) found that the crude protein content of oat silage was lower and NDF content higher than that of barley or triticale silage. Meeske (1999) showed that cutting stage had a major impact on the dry matter content, yield and crude protein content of whole crop oats (Table 1). The protein content of whole crop oats did decline rapidly from 12.1% at the pipe stage to 5.5% at the hard dough stage. In whole crop cereals the digestibility does not decline rapidly with advancing maturity (Baron *et al.*, 1992) as is found in grasses. The declining digestibility in vegetative parts is offset by the increasing contribution of highly digestible grain to the dry weight. The maximum yield from a single harvest will determine the harvest date and this is obtained at the soft dough stage (Meeske, 1999).

The water soluble carbohydrate (WSC) content of cereal crops decreases as the crop matures and grain filling occurs. This may result in low levels of WSC of 2.4 to 4.7 % as reported by Ashbell *et al.* (1987) in wheat, harvested at the soft dough stage. The addition of a lactic acid bacterial inoculant to cereal silages will ensure the efficient utilization of the limited amount of WSC and may therefore improve preservation.

Table 1. The effect of stage of harvesting on the nutritional value (% of DM) and yield (t/ha) of whole crop oats (Cederberg)(Meeske 1999).

Stage	Boot		Bloom			Soft dough			
Date	7/8	14/8	21/8	28/8	4/9	11/9	18/9	28/9	SEM ^g
Composition									
DM	18.2 ^a	20.6 ^{ab}	21.5 ^b	25.5 ^c	28.2 ^d	33.3 ^e	33.1 ^e	46.3 ^f	0.88
Ash	7.8 ^a	7.6 ^a	7.1 ^a	7.1 ^a	6.1 ^b	6.0 ^b	6.3 ^b	6.1 ^b	0.28
CP ^h	12.1 ^a	11.2 ^{ab}	10.2 ^{bc}	9.4 ^c	7.9 ^d	6.9 ^d	7.1 ^d	5.5 ^e	0.40
TDN ⁱ	74.1 ^a	73.4 ^a	72.6 ^a	68.5 ^b	69.5 ^b	65.1 ^c	67.7 ^b	65.1 ^c	0.95
NDF ^g	46.1 ^a	45.6 ^a	48.9 ^b	51.9 ^c	51.1 ^{bc}	51.7 ^c	49.5 ^{bc}	54.5 ^d	0.82
Yield (tonne/ha)									
WM ^h	27.9 ^{ab}	31.0 ^a	31.4 ^a	30.3 ^a	28.6	25.7 ^b	29.3 ^{ab}	17.4 ^c	1.5
DM	5.08 ^a	6.40 ^b	6.72 ^b	7.71 ^c	8.04 ^c	8.48 ^c	9.6 ^d	8.04 ^c	0.32
CP	0.61 ^{ab}	0.71 ^a	0.69 ^{ab}	0.73 ^a	0.64 ^{ab}	0.59 ^b	0.69 ^{ab}	0.44 ^c	0.04
TDN	3.77 ^a	4.69 ^b	4.88 ^b	5.28 ^{bc}	5.59 ^c	5.53 ^c	6.48 ^d	5.23 ^{bc}	0.21

^{abcdef}Means in the same row without a common superscript differ significantly ($P < 0.05$)

^gSEM = Standard error of means, ^hCP = Crude protein,

ⁱTDN = Total digestible nutrients, ^hWM = Wet material

The effect of lactic acid bacterial inoculants on wheat silage has been investigated by several authors. Charmley *et al.* (1996) found that although inoculation had only minor effects on wheat silage composition, there were substantial beneficial effects on animal performance. They found a 10% improvement in body weight gain and an improved feed efficiency when inoculated wheat silage was fed to steers compared to untreated control silage.

In the next paper the hypothesis is tested that the addition of a lactic acid bacterial inoculant to big bale oat silage will improve silage composition, silage intake and milk production.

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The effect of adding an enzyme containing lactic acid bacterial inoculant to big round bale oat silage on intake, milk production and milk composition of Jersey cows.

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Abstract

The ensiling of whole crop oats in wrapped big round bales is becoming popular in South Africa. Low numbers of homofermentative lactic acid bacteria present on whole crop oats prior to ensiling, as well as a low water soluble carbohydrate content, may have a detrimental effect on preservation of whole crop big bale oat silage. The aim of this study was to determine the effect of the addition of an enzyme containing lactic acid bacterial inoculant to big bale oat silage on silage composition, silage intake, milk production and milk composition of Jersey cows. Oats (*Avena sativa*, cv Cederberg), was planted on the 30 th of April 1998 on 2 hectare under dry land conditions in the Western Cape. The crop was cut on the 3 rd of September at the bloom stage, wilted and ensiled in big round bales. The inoculant, Sil-All, was applied during the baling process on half of the bales. Bales were stored for nine months, transported to Outeniqua Experimental farm and fed to Jersey cows in an intake and milk production study. Both the control and inoculated oat silages were well preserved at a DM content of 317 ± 28 and 328 ± 3 and a pH of 4.56 ± 0.05 and 4.52 ± 0.08 respectively. The IVOMD, crude protein, NDF and ADF was 672 ± 8 and 676 ± 17 g/kg DM, 95 ± 9 and 92 ± 9 g/kg DM, 564 ± 9 and 559 ± 3 g/kg DM and 353 ± 7 and 351 ± 11 g/kg DM for the control and inoculated silage respectively. The inoculated silage had a lower level of butyric acid at 5.0 ± 0.5 g/kg DM compared to the 7.2 ± 1.4 g/kg DM of the control oat silage. Twenty two multiparous cows were blocked in pairs and

cows in each pair were randomly allocated to the control or inoculated silage treatment. Whole crop oat silage was fed as only roughage and dairy concentrate was fed at 5.5 kg DM/cow/day. The intake of control and inoculated oat silage was 11.7 and 12.3 kg DM/cow/day ($P=0.09$) respectively. The average daily milk production of the cows fed the inoculated silage was 17.7 kg compared to the 16.7 kg for cows fed the control silage ($P=0.05$). Cows fed the inoculated silage had a significantly ($P=0.01$) lower milk urea nitrogen value, 12 mg MUN/dl compared to that of the cows fed the control silage at 15.1 mg MUN/dl. The addition of an inoculant to big bale oat silage resulted in higher intakes and milk production although only small differences in composition were detected between inoculated and control oat silages.

Keywords: Enzymes; Inoculants; Oat silage; round bale silage; Milk production; Jersey cows

1. Introduction

Big round bale silage is an important feed source for dairy cattle in the Western Cape. The ensiling conditions are not optimal in wrapped big round bales as the material is often not chopped. If unchopped material is ensiled, lactic acid fermentation is weaker and negative effects on feed intake and animal performances are inevitable (Wyss and Jans, 1993). Chopping results in an improved compaction, a more rapid rate of fermentation, increased silage quality and decreased moulding of silage (Nonn and Keller, 1993). Silage making in plastic wrapped big round bales requires pre-wilting to 30% DM to avoid seepage. With 30-40% DM at ensiling, high quality silage cannot be guaranteed and additive application is therefore recommended (Nonn and Keller, 1993). Compaction during the baling process is very important to limit infiltration of air into the bale as bales are not air tight. Sundberg and Thylén (1993) showed that for a dense bale with 180 kg DM per cubic metre at 40% dry matter content, the amount of air entering the bale during a seven week period was slightly more than 300 litres. Fluctuations in temperature, atmospheric pressure and wind cause gas flow into and out of plastic wrapped big bales (Sundberg and Thylén, 1993) even when no visible damage to the plastic layers has occurred. Therefore, conditions to make silage in wrapped big bales are often not optimal.

The quality of silage depends on the quality of the crop at ensiling, type of fermentation, rate of pH decrease, moisture content of the crop and the maintaining of anaerobic conditions. The rate of pH decrease is determined by the level of water soluble carbohydrates (WSC) and the epiphytic bacteria present on the crop prior to ensiling (McDonald *et al.*, 1991; Rooke, 1990). According to Jones *et al.* (1998) the WSC content of whole crop oats can be as low as 55 g/kg DM at the soft dough stage. If sufficient lactic acid bacteria are not present on the crop at time of ensiling, a slow rate of pH decrease will result. The pH should drop rapidly below 5 to prevent the growth of *Clostridia tyrobutyricum* (McDonald *et al.*, 1991). These are anaerobic bacteria that break down protein and produce butyric acid. This will result in silage with a lower palatability and lower intake by animals. Anaerobic conditions are reached within 24 hours after bales are wrapped. The use of a lactic acid bacterial inoculant may improve the fermentation (Moshtaghi Nia and Wittenberg, 1999) of silage and animal production. The aim of this study was to determine the effect of adding a lactic acid bacterial inoculant to big bale oat silage on silage composition, intake, milk production and milk composition of Jersey cows.

2. Material and methods

2.1. Crops and silage

Oats (*Avena sativa*, cv Cederberg) was planted on the 30 th of April 1998 on 2 hectare under dry land conditions on the farm near Bredasdorp (20° 02' S, 34° 30' E; altitude 153m) in the Western Cape. The pH (KCl) of the soil was 5.5, phosphorous 42 ppm and potassium 230 ppm. Before planting 175 kg of 3:2:0(30%: 18%N and 12%P) was applied per hectare. Oats was planted with a 12 metre Amazon planter at 120 kg/ha. Seven weeks after planting 125 kg Kysan (Limestone ammonium nitrate plus sulphur, 27% N, 3.7% Ca and 3.5% S) was applied per hectare. On the 3 rd of September, the whole crop oats was cut at the bloom stage, wilted to a dry matter content of 33%, baled with a Krone KR130 baler and wrapped with a Kverneland wrapper (UN 7558) using Silawrap film (750mm).

2.2. Application of the inoculant

The inoculant, Sil-All (Supplied by Alltech Biotechnology Pty. Ltd.) containing *Lactobacillus plantarum*, *Streptococcus faecium* and *Pediococcus acidilactici* together with the enzymes, cellulase, hemicellulase and amylase was applied during the baling process with a pump and sprayer system at 10 g per ton of fresh material on half of the bales. Bales were stored for nine months, transported to Outeniqua Experimental farm and fed to Jersey cows in an intake and milk production study.

2.3. Animals and diets

Twenty two multiparous cows were blocked in pairs according to milk production (previous 4 weeks), days in milk, lactation number, liveweight and condition score. Within each pair, cows were randomly allocated to the control or inoculated silage treatment. The experimental period consisted of an adaptation period of 10 days followed by a measurement period of 21 days. Silage was chopped with a Selbourne mixer Wagon and was fed individually to cows. Dry matter intake was determined on a daily basis. Cows were milked two times per day at 07:00 and 15:30. Three kilograms of a commercial dairy concentrate (230 g CP/kg concentrate and 12 MJ ME/kg concentrate) was fed after each milking to all cows. Silage was fed individually to cows twice daily at 8:00 and 16:30. Cows had access to silage from 8:00 to 12:00 and from 16:30 to 20:30. Silage left over in the morning was removed and weighed. The amount of silage fed to each cow was adjusted every second day to ensure availability of silage was not limiting (10% residue was allowed). Cows were in a small rest camp with access to water only from 12:00 to 15:30 and from 20:30 to 07:00 the next morning. Cows were weighed at 08:00 on two consecutive days at the start and the end of the experimental period.

2.4. Sample preparation and chemical analyses

Samples of control silage, inoculated silage and concentrate were taken on Monday, Wednesday and Friday during the measurement period. These samples were pooled for each week and frozen

at -10°C. Composite samples were dried at 60°C for 72 hours. Water soluble carbohydrate (WSC), pH and lactic acid (LA) content were determined on filtrates of 40 g of frozen sample added to 360 ml of distilled water, homogenized for 3 minutes with a stomacher. WSC were determined by the phenol-sulphuric acid method according to Dubois *et al.* (1956) and LA was determined by the colorimetric method of Barker and Summerson (1941). Volatile fatty acids (VFA) were determined with a Carlo Erba 4200 gas chromatograph with flame ionisation detector with a 2.35m x 3mm stainless steel column packed with 10% SP 1200 containing 1% ortho-phosphoric acid (H_3PO_4). The column was conditioned for 48 hours at 165°C with a nitrogen carrier gas flow of 40 ml per minute. *In vitro* organic matter digestibility (IVOMD) was determined according to Tilley and Terry (1963). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined according to Van Soest *et al.* (1991). Total nitrogen was determined by the Kjeldahl method. The ammonia nitrogen content of silage was determined by homogenizing 50 g of silage in 250 ml of a 0.1N H_2SO_4 solution for three minutes. The homogenate was filtered through Whatman no 4 filter paper and the ammonia content in the filtrate was determined by distillation using a Buchi 342 apparatus and a Mettöhlm 655 Dosimat with a E526 titrator according to AOAC (1984). This method is based on the method of Pearson and Muslemuddin (1968) to determine volatile nitrogen.

A composite milk sample of the morning and afternoon milking was taken on a weekly basis during the experimental period. The protein, butterfat and milk urea nitrogen value of the milk were determined with a 605 Milko Scan analyser, a mid-range infrared spectrophotometer, according to the International IDF standard 141B (1996).

2.5. Statistical analysis

Statistical analysis was done using the paired t-test (McCall, 1970). A two tailed test was performed on pairs. The pairs were randomly selected and it was assumed that the population is normally distributed. When the observed t-value was greater than 1.812, the hypothesis that the means for treatments were similar was rejected, and, therefore a significant ($P<0.05$) difference between treatments was found. Significance was declared at $P<0.05$, unless otherwise indicated.

3. Results

3.1 Chemical composition of the silage

Seventy two bales were harvested with an average weight of 690 ± 38 kg. The yield was 6.5 ± 0.24 ton dry matter per hectare ($n = 4$). The composition of control oat silage, inoculated oat silage and that of the dairy concentrate fed to the Jersey cows is given in Table 1.

Table 1

The chemical composition (g/kg DM \pm SD) of big round bale oat silage with or without the addition of an enzyme containing lactic acid bacterial inoculant, and the composition of the dairy concentrate fed to Jersey cows.

	Silage		Concentrate
	control	inoculant	
n	3	3	3
Dry matter	317 ± 28	328 ± 30	898 ± 3
Organic Matter	914 ± 8	915 ± 12	918 ± 6
Neutral detergent fibre	564 ± 9	559 ± 3	223 ± 7
Acid detergent fibre	353 ± 7	351 ± 11	80 ± 3
Calcium	5 ± 0.1	5 ± 0.1	14 ± 1
Phosphorous	3 ± 0.1	3 ± 0.1	8 ± 0.1
IVOMD ^a	672 ± 8	676 ± 17	855 ± 9
Crude protein	95 ± 9	92 ± 9	258 ± 9
NH ₃ -N (g/kg TN ^b)	87.6 ± 9.2	87.8 ± 11	
pH	4.56 ± 0.05	4.52 ± 0.08	
WSC ^c	49.4 ± 14.5	60.5 ± 9.3	
Lactic acid	24.4 ± 3.3	28.2 ± 11.2	
Acetic acid	6.4 ± 1.3	6.4 ± 0.7	
Propionic acid	0.24 ± 0.07	0.19 ± 0.09	
N-butyric acid	7.2 ± 1.4	5.0 ± 0.5	

^a *In vitro* organic matter digestibility; ^b Total Nitrogen; ^c Water soluble carbohydrate.

Addition of the inoculant resulted in lower levels of n-butyric acid compared to untreated silage. This indicates that the rate in pH decrease may have been faster in the inoculated silage.

3.2. Feed intake and milk production

Feed intake, milk production and milk composition of Jersey cows is presented in Table 2.

Table 2

Feed intake, liveweight change, condition score, milk production and milk composition of Jersey cows fed big round bale oat silage with or without the addition of an enzyme containing lactic acid bacterial inoculant.

	Control	Inoculant	P-value	T-value
<i>Intake</i>				
Silage (kg DM/day)	11.7	12.3	0.09	1.55
Concentrate (kg DM/day)	5.5	5.5		
Total (kg DM/day)	17.2	17.8	0.09	1.55
Dry matter intake (g/kg liveweight)	45.1 ^a	49.6 ^b	0.003	5.53
<i>Liveweight (kg)</i>				
Start of study	366 ^a	344 ^b	0.01	-3.32
End of study	393 ^a	373 ^b	0.02	-2.30
Change during period	+27	+29	0.38	0.30
<i>Condition score (1-5)</i>				
Start of study	2.27	2.21	0.29	-0.94
End of study	2.05	2.00	0.17	-1
<i>Milk production (kg/day)</i>				
kg milk/cow/day	16.7 ^a	17.7 ^b	0.05	1.89
kg milk/100 kg LW/day	4.43 ^a	4.99 ^b	0.01	3.41
kg FCM/cow/day	19.2	19.9	0.11	1.35
kg FCM/100 kg LW/day	5.07 ^a	5.62 ^b	0.01	3.36
<i>Milk composition</i>				
Butterfat (g/kg)	50.6	49.6	0.26	-0.87
Protein (g/kg)	34.7	34.6	0.42	-0.51
Milk urea nitrogen (mg/dl)	15.1 ^a	12.0 ^b	0.01	-3.07

^{a,b}Means with different superscripts in the same row differ significantly (P<0.05)

The inoculant treatment of big round bale silage resulted in an increase in milk production of 1 kg per cow per day. The intake of inoculated silage tended to be higher than that of the control silage. The average liveweight of cows in the control group was significantly higher than that of the cows in the inoculated silage group at the start of the study. Therefore, total DM intake was expressed in g/kg liveweight in Table 2. No significant differences were found in the butterfat and the protein content of milk from cows fed the inoculated or control silages.

4. Discussion

4.1. Effect of treatments on silage composition

The variation between the weekly pooled silage samples were high, as is shown by the large standard deviations (Table 1). This may have occurred as a result of heterogeneity in microbial activity in the bales (Jonsson *et al.*, 1990). The inoculated big round bale oat silage did not have a significant lower pH and a higher lactic acid content compared to control silage, as was found by Moshtaghi Nia and Wittenberg (1999) for inoculant treated big bale whole crop barley silage.

The untreated and inoculant treated oat silages in our study were well preserved at a pH of 4.56 and 4.52 and a DM content of 317 and 328 g/kg silage respectively. According to Weissbach (1996) the pH of silage should be 4.53 to ensure effective preservation when the DM content of a crop is 320 g/kg silage. Although the growth of most acid-tolerant clostridia is inhibited by a pH just below 5 (Jonsson, 1991), Jonsson *et al.* (1990) found that a DM of 350 g/kg in big bale grass silage was not high enough to give an acceptable reduction of clostridial activity. Jonsson *et al.* (1990) has shown that wilting and the use of additives increased the quality of big bale grass silage by a reduced pH, lower ammonia-N, butyric acid and clostridial spores. The growth of *Clostridia tyrobutyricum* was not completely inhibited in silages treated with additives, but butyric acid production and sporulation were reduced (Jonsson *et al.*, 1990). In our study, the addition of a lactic acid bacterial inoculant to big bale oats at ensiling, did result in lower levels of N-butyric acid compared to the untreated control silage (Table 1), indicating a lower clostridial activity.

The NDF and ADF content of inoculant and enzyme treated big bale oat silage did not differ from that of the untreated control silage (Table 1). This indicates that the enzymes added did not cause a measurable amount of fibre breakdown, as was found by Moshtaghi Nia and Wittenberg (1999) when ensiling big bale barley whole crop with the addition of an inoculant or enzyme containing. The NDF and ADF content of 564 and 353 g/kg DM respectively for oat silage in this study compares well with the 525 g NDF/kg DM and 341 g ADF/kg DM found by Moshtaghi Nia and Wittenberg (1999) for big bale whole crop barley silage. The WSC content of the control and inoculated silage was 49.4 and 60.5 g/kg DM, respectively, after 9 months of ensiling which indicates that WSC did not limit the rate of pH decrease and preservation in this study. When whole crop oats is harvested at the soft dough stage, the WSC content can be as low as 55 g/kg DM prior to ensiling (Jones *et al.*, 1998) which may then limit the rate of preservation.

Only three pooled big bale oat silage samples were analysed for each of the treatments. With more frequent sampling of silages, differences between treatments in pH, lactic acid and WSC content may have been more pronounced. The sampling of individual bales before chopping would have given a better comparison and more replicates for each treatment.

Both the control and inoculated silages were stable when exposed to air and no heating of silage occurred during the feeding out of silage. This can be explained by the acetic acid and N-butyric acid found in the silages which would inhibit the growth of yeasts and moulds (Weissbach, 1996). Moshtaghi Nia and Wittenberg (1999) found that adding a microbial inoculant to big bale whole crop barley silage improved the aerobic stability of the silage compared to control silage. The DM and WSC content of the big bale barley silage was high at 472 g DM/kg and 110 g WSC/kg DM, respectively, creating a high risk for aerobic deterioration. The high dry matter would increase the risk of air infiltration, creating favourable conditions for the growth of yeasts and moulds in the presence of sufficient WSC.

4.2. Milk production

The milk production of cows that were fed the inoculated round bale oat silage was significantly

higher than that of cows receiving the control silage. This coincided with a higher intake of inoculated silage compared to the control silage. The chopping of the big bale silage with a mixer wagon before feeding may have contributed to the high total DM intakes of 4.5 and 4.9% of liveweight for the control and inoculated silage diets, respectively.

The addition of the inoculant resulted in a significant lower milk urea nitrogen (MUN) content compared to the control silage, suggesting a more efficient crude protein utilization in the inoculated silage. The lower n-butyric acid found in the inoculated silage compared to the control silage, suggested a lower clostridial activity and therefore less proteolysis could be expected. The ammonia-N as percentage of the total nitrogen for the control and inoculated silages were, however, similar. The inoculant had no significant effect on butterfat and protein content of the milk. The MUN values would suggest that the level of protein feeding was adequate. According to Melendez *et al.* (2000), the MUN value should be between 12 and 18 mg MUN/dl milk.

5. Conclusions

The addition of a lactic acid bacterial inoculant with enzymes to big bale oat silage resulted in an increased silage intake of 0.5 kg DM /day and an increased milk production of 1 kg/day ($P=0.05$) by Jersey cows compared to the untreated control silage. The chemical composition of big bale oat silage was however not greatly affected by the addition of the inoculant. The inoculant did not affect the aerobic stability of big bale oat silage, both control and inoculated silages were stable during aerobic exposure.

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Chapter 7

General discussion.

1. Introduction

The effect of lactic acid bacterial inoculants on silage made from tropical grasses, lucerne, sorghum, whole crop maize and whole crop oats have been presented in previous chapters. The addition of a lactic acid bacterial inoculant to tropical grasses, lucerne and sorghum did result in a more rapid rate of preservation and improved silage quality.

2. Ensiling of tropical grasses

The addition of inoculants to tropical grasses (*Digitaria eriantha* and *Eragrostis curvula*) has been shown improve the fermentation dynamics during ensiling. The improved preservation of *D. eriantha* did also result in an improvement of *in vivo* organic matter digestibility and an increased silage intake by Merino rams. In both studies the lactic acid content of inoculated silage was increased, the pH was lower and the acetic acid, butyric acid and ammonia-nitrogen as percentage of total nitrogen was lower than that of the control silage. Preservation of tropical grass silage made without any additive was poor.

The addition of the inoculant did not have a detrimental effect on the aerobic stability of tropical grass silages. Although the acetic acid content of inoculated tropical grass silage was lower than that of the control silage, inoculated silage still contained more than 5.9g acetic acid/kg DM. Weissbach (1996) evaluated 54 farm scale grass silages and found that when the acetic acid content of silages was lower than 3g/kg DM, 64% of the silages were very unstable (<3 days).

Domingues and Hardy (1988) ensiled *Cynodon dactylon* at two stages of maturity and added three levels of molasses in laboratory silos. They concluded that *Cynodon dactylon* could be ensiled at 37 days of regrowth without the adding of molasses. The composition of the silage however does indicate poor preservation (DM=26%, pH= 4.9, lactic acid= 1.29% of DM and NH₃-N % of total nitrogen 19.8%).

The addition of a efficient lactic acid bacterial inoculant to tropical grass at ensiling will ensure

optimal use of the limited amount of water soluble carbohydrates that are available for fermentation. This will increase the rate of preservation and improve silage quality and intake of silage.

3. Ensiling of lucerne

Conserving lucerne as silage may be a better option than conserving lucerne as hay (Etheridge *et al.*, 1992). Field losses are potentially less with silage than with hay. Well-preserved lucerne silage has at least as high a feeding value as well made lucerne hay (Waldo, 1977). Cows fed lucerne silage ate more and produced more milk than cows fed lucerne hay (Nelson and Satter, 1992).

In our study, the addition of a lactic acid bacterial inoculant with enzymes to lucerne did improve preservation, decreased proteolysis and did result in efficient utilization of the limited amount of water soluble carbohydrates. The addition of molasses did also improve the lucerne silage but was less effective than the inoculant. Seale *et al.* (1986) found that if insufficient sugar is present in the original crop, then lactic acid bacteria in an inoculant will not be able to produce enough lactic acid to lower the pH to an acceptable level. The lucerne was ensiled in laboratory silos and the WSC prior to ensiling was 49g/kg DM. In our study the WSC content was similar to that reported by Seale *et al.* (1986), at 47.5g/kg DM prior to ensiling and this was adequate for effective preservation. The enzymes, cellulase, hemicellulase and amylase may have hydrolysed some of the plant cell walls making more carbohydrates available to the lactic acid bacteria. The NDF content of the inoculated lucerne silage did however not differ from the control lucerne silage. Tengerdy *et al.* (1991) did show that ensiling lucerne with lactic acid bacteria and enzymes, increased fibre digestibility only in direct cut lucerne silage but not in wilted lucerne silage. The additive did result in a rapid, controlled homolactic fermentation of both direct cut and wilted lucerne silage.

The dry matter content of lucerne silage has a major impact on fermentation and silage quality. Luchini, *et al.* (1997) evaluated lucerne taken from 21 bunker silos and found that the DM was 36.8%; pH, 4.84; lactic acid, 3.67%; acetic acid, 2.87%; butyric acid, 1.04%; NH_3N % of total N, 13.1%. The ammonia nitrogen content and butyric acid content does indicate that fermentation

of lucerne silage can be improved by increasing the rate and efficiency of fermentation. Butyric acid was not found in lucerne silages with a DM content of above 45% compared to the 1 to 5% butyric acid found in lucerne silage at a DM content of 25%.

The effect of inoculating lucerne silage on animal performance was not evaluated in our study. Kung *et al.* (1987) and Thomas *et al.* (1983) found milk yield of cows increased when inoculated lucerne was fed compared to untreated lucerne silage, while Ahrens *et al.* (1981), Grieve *et al.* (1982) and Kent *et al.* (1989) found no effect on milk production.

Our study did show improved preservation when adding an inoculant with enzymes to big bale lucerne silage and similar results have been found by Ciotti *et al.* (1993).

4. Ensiling of forage sorghum

The forage sorghum Dekalb FS 2 did have a high energy value (IVOMD= 67.6%) before ensiling at the soft dough stage. The addition of inoculants did improve the fermentation dynamics during ensiling but had a detrimental effect on the aerobic stability of sorghum silages.

5. Ensiling of maize

The evaluation of different maize hybrids as silage crops clearly showed differences in energy content, predicted intake and milk production potential. More emphasis should be placed on the nutritional value of maize hybrids when choosing a hybrid as silage crop.

Good quality maize silage can be made without using any additive. Both laboratory studies showed very little effect of lactic acid bacterial inoculants on the fermentation dynamics during ensiling. Inoculation did however improve the palatability of maize silage and higher intakes of inoculated maize silage by sheep and dairy cows were recorded. Lambs fed inoculated maize silage tended to weigh more after a 60 day growth period compared to that of lambs fed the control silage. Maize silage is an excellent feed source for growing lambs as indicated by the

average daily gains of $239 \pm 26\text{g}$ and $255 \pm 44\text{g}$ for lambs fed diets consisting of either 60% control or inoculated maize silage. Milk production of Jersey cows did not increase significantly when inoculated maize silage was fed.

6. Ensiling of whole crop oats

Although the inoculation of big bale oat silage did result only in small changes in the composition of the silage, milk production of cows fed inoculated silage was increased by 1 kg/day compared to that of cows fed control oat silage. Oat silages were stable when exposed to air.

7. Laboratory silos versus commercial silos

Laboratory silos have been used in many studies to evaluate silage inoculants. Laboratory experiments are useful to study the principles of the ensiling process, but differences in air influence, gas atmosphere and temperature do not allow direct translation of laboratory results to farm scale silages (Spoelstra, 1990). Wilson and Wilkins (1972) stated that laboratory silos have considerable potential in research, allowing a wider variety of crops and treatments to be studied that would have been possible on a larger scale.

Under commercial ensiling conditions in South Africa where farmers seldom have access to the services of a contractor, it may take from 1 to 4 weeks to fill and seal a silage bunker, depending on the size of the bunker, silage-making equipment and available labour. Delayed sealing of the silage bunker will result in higher temperature in the ensiled material, a lowering of the water soluble carbohydrate content, loss of dry matter and a lower nutritional value of resulting silage. Less WSC will then be available for the lactic acid bacteria to produce lactic acid (Woolford, 1990; Keller *et al.*, 1996).

A time delay in the sealing of a bunker silo may be more detrimental when ensiling tropical grasses and lucerne that have low levels of water soluble carbohydrates compared to when maize is ensiled. Under aerobic conditions lactic acid bacteria utilize carbohydrates and produce acetic

and lactic acid, acetone, carbon dioxide and hydrogen peroxide. Acetic acid bacteria grow under aerobic conditions, they utilize lactic acid and produce acetic acid (Courtin and Spoelstra, 1990). Higher levels of acetic acid can therefore be expected in bunker silos compared to silage made in laboratory silos as air does penetrate into the bunker silo and filling and sealing is more time consuming. In the study of Meeske *et al.* (1999), silage made of *Digitaria eriantha* in laboratory silos without the addition of an inoculant did have a lower acetic acid (9.9 g/kg DM) content and contained no butyric acid compared to silage made of the same material in tower silos that contained 15.2 g acetic acid/kg DM and 7.5g butyric acid/kg DM.

Lucerne silage made in laboratory silos (Chapter 3) without any additive contained 7.3% lactic acid, 3.9% acetic acid and no butyric acid compared to the 3.08% lactic acid, 5.06% acetic acid and 0.28% butyric acid found in big round bale silage.

The maize silage made in 210 litre drums contained three times more acetic acid than maize silage made in laboratory silos (Meeske and Basson, 1998). The difference between laboratory and bunker maize silage was even greater in the study of Meeske *et al.* (Chapter 4 of this thesis) where only traces (0.08 and 0.06 g/kg DM) of acetic acid were found in laboratory made silage compared to 27 and 20.1 g acetic acid/kg DM for control and inoculated maize silage made in a bunker silo. In both the maize silage studies the lactic acid content of laboratory maize silage was higher than that of maize silage made on larger scale.

Laboratory silos do create more favourable ensiling conditions compared to practical ensiling conditions on the farm. If an additive does have a positive effect on preservation of plant material ensiled in laboratory silos, the chance of showing a positive effect under commercial ensiling conditions is good. On the other hand, if evaluating an additive in a laboratory study does not show any positive effects, a positive effect when making silage under less favourable commercial conditions is not ruled out. Ely *et al.* (1981) suggested that the beneficial effect of an inoculant on nutrient recoveries may be greater under less controlled ensiling conditions than found in laboratory silos. Final evaluation of silage additives should be done under practical conditions that prevail on the farm.

8. Aerobic deterioration of silage

Aerobic deterioration is a microbiological process carried out by aerobic micro-organisms that cannot proliferate in the anaerobic environment of a sealed silo (Honig and Woolford, 1980).

Aerobic deterioration is always accompanied by a loss of residual sugars, increase in temperature and the evolution of ammonia and carbon dioxide (Woolford, 1990). Poor quality silage is always very stable in air, has a high pH, high contents of butyric acid and ammonia, low contents of lactic and acetic acid. Butyric acid and higher fatty acids, together with ammonia, act as very effective preservatives (Woolford, 1990). Aerobic deterioration of silage is undesirable because of the high nutrient losses associated with it. Intake of deteriorated silage is restricted or it may even be rejected by animals, if it is consumed there is a health risk to the animal (McDonald *et al.*, 1991).

Carbohydrate rich silages such as maize and sorghum are more prone to aerobic deterioration than lucerne or tropical grass silages where carbohydrates are virtually depleted after the ensiling process. In maize silage, acetic acid bacteria and yeast initialize aerobic deterioration (Spoelstra *et al.*, 1988). Acetic acid bacteria first oxidize ethanol to acetic acid and water. Consequently when the aerobic bacteria (proteolytic species of *Bacillus*) increase to 10^5 to 10^6 per gram of silage, oxidation of lactic acid to acetic acid and CO_2 takes place which results in an increase in pH and a rise the temperature of the silage. The temperature increase then speeds up the deterioration of the silage and mould starts to grow (Courtin and Spoelstra, 1990).

Meeske and Basson (1998a) found that untreated and inoculated *Eragrostis curvula* silage made in 1.5 litre laboratory silos was stable when exposed to air for five days. The acetic acid content of the control and inoculated silage was high at 4.33 and 4.00 % of DM and the WSC content was very low at 0.65 and 0.85% for the respective treatments.

When the *Digitaria eriantha* silage was exposed to air for five days, the pH increased from 4.87 to 6.75 in the control silage and from 3.9 to 4.51 in the inoculated silage (Meeske and Basson,

1998b). The inoculated silage was more stable than the control silage when exposed to air, as indicated by the lower production of CO₂, slower increase in pH and no mould detected in the inoculated silage compared to the control silage. Improved aerobic stability of silage can be expected when lactic acid bacteria are added and the pH is lowered quickly (McDonald *et al.*, 1991). Undissociated lactic acid and acetic acid do have an inhibitory effect on yeast growth (Moon 1983; Courtin and Spoelstra, 1990).

Lucerne silage made in laboratory silos (Chapter 3) was stable when exposed to air and inoculation did not effect the aerobic stability of lucerne silages. The low levels of residual WSC in the silage as well as relative high levels of acetic acid may explain the aerobic stability of lucerne silage. Kung *et al.* (1991) also found that both untreated and inoculated lucerne silages were stable when exposed to air.

Big bale whole crop oat silage was stable when exposed to air and no heating occurred during the feeding period. The control and inoculated silages did however contain 0.72% and 0.5% butyric acid respectively and both silages contained 0.64% acetic acid.

Driehuis *et al.* (1996) increased the aerobic stability of maize silage by the inoculation with *Lactobacillus buchneri*. This lactic acid bacteria is heterofermentative and produces lactic acid, acetic acid and propionic acid. Driehuis *et al.* (1999) did however find that *L. buchneri* was more effective under laboratory than under farm ensiling conditions and that feed intake and performance of dairy cows was not affected by use of the bacteria.

Inoculation did not affect aerobic stability of maize silage in our first study and improved the aerobic stability in the second study.

O' Kiely and Muck (1992) studied the aerobic stability of lucerne and maize silage and concluded that aerobic stability was not related to silage DM, pH, yeast numbers or glucose addition at ensiling. Stability appeared to be due to the presence of inhibitors produced during ensiling. The unstable silages were lower in 2,3-butanediol than most other silages. This compound can be

produced under anaerobic conditions by lactic acid bacteria, enterobacteria and bacilli (McDonald *et al.* 1991)

The aerobic stability of forage sorghum silage ensiled at the soft dough stage was reduced by the addition of a bacterial inoculant (Meeske *et al.*, 1993). This may occur when the silage contains a high level of water soluble carbohydrates (60 g WSC/kg DM) and lactic acid (70 g LA /kg DM) in the presence of high numbers of lactate assimilating yeasts (Meeske *et al.*, 1993). The positive effect of the inoculant on the ensiling of sorghum at the soft dough stage was largely lost due to the detrimental effect of the inoculant on the aerobic stability of the silage. The pH of inoculated silages increased, lactic acid and WSC content decreased and the IVOMD dropped from 65.6 to around 60.7%. The IVOMD of the control silage was 64.3% after 5 days of aerobic exposure. Because the ensiling of sorghum was adequate and the adverse effect of lactic acid bacterial inoculants on the aerobic stability of the silage the need for inoculants on such crops may be questioned. Saunderson (1993) ensiled sorghum at the soft dough stage with or without the addition of a lactic acid bacterial inoculant in laboratory silos. When exposed to air for 7 days, both control and inoculated silages were very unstable. During aerobic exposure the number of yeasts increased greatly, pH increased and WSC decreased from 6.7 to 1.1%. Black *et al.* (1980) did not find a positive effect of silage additives on sorghum silage.

Weinberg *et al.* (1991) ensiled wheat at the early milk, milk and dough ripening stage in 1.5 litre glass jars and found that all silages were stable when exposed to air for 5 days.

Many studies have shown that silages made in bunker silos under commercial farm conditions often contain less lactic acid and more acetic acid than silages made in small scale laboratory silos. Silages made in bunker silos may therefore be more stable when exposed to air than silages made in laboratory silos. On the other hand yeast and mould will have a longer time to multiply when bunker silage is made as it takes longer achieve anaerobic conditions. This may then result in higher numbers of yeast and mould that will start growing when air enters the silage. When the effect of silage additives on aerobic stability of silage is determined the final evaluation should include studies on large scale bunker silages.

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